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ABSTRACT

The report recommends government actions that should be taken concerning (1) the coordination of information dissemination and research related to the< effects of pesticides; (2) the control or elimination of the use of specific persistent pesticides in the United States, and the development of standards for the exposure of humans to insecticides in food, water, and air; and (3) regulation and legislation concerning pesticide development, testing and use. These recommendations are based on the extensive reviews of the literature included as Part 2 of the report. The topics reviewed were: (1) uses and benefits; (2) contamination; (3) effects on non-target organisms other than man; (4) effects on man, (5) carcinogenicity; (6) interraction (in target and non-target organisms); (7) mutagenicity; and (8) teratogenicity (abnormalities of pre-natal origin). Bibliographies are included in each review. Reviews indicate needed research as well as available data. Possible alternative means of pest control are considered in reviews 1, 2, and 3. (AL)

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Report of the Secretary's Commission on Pesticides and Their Relationship to Environmental Health

Parts I and II

SE 010 121

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
December 1969

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Report
# of the
Secretary's Commission
on
Pesticides
and
Their Relationship
to
Environmental Health

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
December 1969

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DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

December 5, 1969

Dear Mr. Secretary:

The complete report of your Commission on Pesticides and Their Relationship to Environmental Health (Part I & Part II) is included herein.

Part I, which I submitted to you on November 11, 1969, contains the Commission's unanimous recommendations along with summaries of the reports of four Subcommittees to the Commission.

Part II contains the complete reports and conclusions of four Subcommittees and the four Advisory Panels to the Commission, upon which findings the Commission based its recommendations. Over 5000 references to published or ongoing scientific research were reviewed and evaluated. Since each report was prepared by the membership of the Subcommittee or Advisory Panel involved with the particular subject under review, these reports by themselves do not necessarily reflect the unanimous opinion of the Commission's entire membership, although each Commission member has reviewed all drafts of all reports. However, the recommendations of the Commission were adopted unanimously.

On behalf of the Commission, its entire staff, and speaking for myself, I would like to thank you for the superb support you and your Department have given to the Commission. We hope that our recommendations will be considered for early implementation by all Departments concerned with the use of pesticides.

Sincerely yours,

Emil M. Mrak
Chairman
Secretary's Commission on Pesticides
and Their Relationship to Environmental
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Honorable Robert H. Finch
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Part I

Commission Recommendations on Pesticides

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

November 11, 1969

Dear Mr. Secretary:

Attached are the unanimous recommendations of your Commission on Pesticides and Their Relationship to Environmental Health. As you know, the reports of the Commission, eight in all, are in the process of publication as you have directed.

These recommendations are arranged in an action priority order as seen by the Commission; however, this order has no significance as to their relative importance. In my own view the recommendations support the following principles:

1. Chemicals, including pesticides used to increase food production, are of such importance in modern life that we must learn to live with them;
2. In looking at their relative merits and hazards we must make individual judgments upon the value of each chemical, including the alternatives presented by the non-use of these chemicals. We must continue to accumulate scientific data about the effects of these chemicals on the total ecology; and
3. The final decision regarding the usage of these chemicals must be made by those governmental agencies with the statutory responsibilities for the public health, and for pesticide registration.

On behalf of the Commission and all of the staff assigned to help the Commission, I want to express our thanks for the support you have given us in carrying out this task. We hope that our efforts will be helpful to you in carrying out your awesome responsibilites.

Sincerely yours,

Emil M. Mrak
Chairman
Secretary's Commission on Pesticides and
Their Relationship to Environmental Health

Honorable Robert H. Finch
Secretary
Department of Health, Education,
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Washington, D.C.

Enclosures

INTRODUCTION

Our society has gained tremendous benefits from the usage of pesticides to prevent disease and to increase the production of foods and fibers. Our need to use pesticides and other pest control chemicals will continue to increase for the foreseeable future. However, recent evidense indicates our need to be concerned about the unintentional effects of pesticides on various life forms within the environment and on human health. It is becoming increasingly apparent that the benefits of using pesticides must be considered in the context of the present and potential risks of pesticide usage. Sound judgments must be made.

The Secretary's Commission on Pesticides and Their Relationship to Environmental Health was appointed in April 1969 and charged with the responsibility of gathering all available evidence on both the benefits nad risks of using pesticides, evaluating it thoroughly, and reporting their findings and recommendations to Secretary Finch. After carefully reviewing all available information, the Commission has concluded that there is adequate evidence concerning potential hazards to our environment and to man's health to require corrective action. Our Nation cannot afford to wait until the last piece of evidence has been submitted on the many issues related to pesticide usage. We must consider our present course of action in terms of future generations of Americans and the environment that they will live in.

The Commission's unanimously approved recommendations, developed after careful evaluation of all available evidence, are presented in this document. Part II of this report currently is in preparation for printing and will be made available in the near future. This represents the review of over 5,000 references to scientific research, and will contain a full presentation of each Sub-Committee's Report to the Commission, including special Advisory Panel Reports on carcinogenesis, interaction, mutagenesis, and teratogenesis. We must consider the total problem of pesticide usage not only in the context of what is presently known but also in the context of the many unknowns still to be determined. Some of these unknowns may never be precisely determined. Corrective action is recommended now to prevent further environmental contamination from pesticide residues and to protect the health of man.

COMMISSION RECOMMENDATIONS

Recommendation 1:

Initiate closer cooperation among the Departments of Health, Education, and Welfare, Agriculture, and Interior on pesticide problems through establishment of a new interagency agreement.

The registration of pesticides is now vested only in the Secretary of Agriculture under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). The regulations implementing FIFRA state that the purpose of the act is "to protect the public health before injury occurs rather than to subject the public to dangers of experimentation and take action after injury."[1] However, the present interagency agreement requires the Secretaries of DHEW and USDI to produce scientific evidence clearly demonstrating a present hazard to health or to the environment in order to remove from registered use or prevent the registration of any specific pesticide. In regard to health protection, the burden of proof should rest upon the manufacturer to demonstrate to the Secretary of HEW that appropriate tests do not produce untoward effects upon two or more species of mammals which might indicate a hazard to health. Such a procedure is entirely in keeping with the purpose of the act as stated above.

A new interagency agreement is needed to strengthen cooperative action among the Departments of HEW, USDA, and USDI to protect public health and the quality of the environment from pesticide hazards. Approval by the Secretaries of DHEW and Interior as well as Agriculture should be required for all pesticide registrations. Pesticide uses deemed by any of the three Secretaries to be hazardous should be restricted or eliminated.

The agreement should further require a continuous review of new scientific information on pesticides now in use, with a formal review made 2 years after initial registration and subsequent formal reviews by the three agencies at 5-year intervals.

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[1] C.F.R., Title 7, Cr. 11, Sec. 362.106(d) (1).

Such an agreement and the closer interagency cooperation it would produce would have other distinct advantages:

- It would call attention to evidence suggesting concern and expedite appropriate action;
- It would encourage cooperative approaches to public education, applicator training, research on the biological effects of a pesticide, and promote the development and use of improved methods of pest control; and
- It would provide a mechanism for focusing the concerns and skills of each agency and to coordinate action on pesticide problems.

If the objective of providing to the Secretary of DHEW the authority to meet his responsibility for control of health hazards of pesticides cannot be attained by a new interagency agreement, it will be necessary to amend the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA).

Recommendation 2:

Improve cooperation among the various elements of the Department of Health, Education, and Welfare which are concerned with the effects of pest control and pesticides.

The diversified and significant responsibilities associated with the Department of Health, Education, and Welfare pesticide and pest-control activities lack sufficient coordination and direction. Several segments of DHEW have direct and implied responsibilities for proteciton of public health in relation to the use of pesticides. The problem of achieving cooperation appears to be intensified by recent reorganizations. Mechanisms should be developed to assure exchange of information between all pertinent segments of DHEW. There is a need for reappraisal of the vector control activities, educational programs, research responsibilities, monitoring, State aid programs, and other activities involving pesticides.

Recommendation 3:

Eliminate within two years all uses of DDT and DDD in the United States excepting those uses essential to the preservation of human health or welfare and approved unanimously by the Secretaries of the Departments of Health, Education, and Welfare, Agriculture, and Interior.

The uses of DDT and DDD as pesticides should be limited to the prevention or control of human disease and other essential uses for which no alternative is available. Such uses should be clearly identified and individually evaluated in relation to human hazard from exposure, movement in the natural environment concentration in the food chains of the world, and other environmental considerations. Unanimous approval by the Secretaries of DHEW, USDA, and USDI (who in turn are expected to call on Federal, State and private experts for advice) would provide for Identification of essential uses and assure that such approval will be based on sound judgment.

Abundant evidence proves the widespread distribution of DDT and its metabolites (principally DDE) in man, birds, fish, other aquatic organisms, wildlife, soil, water, sewage, rivers, lakes, oceans, and air. Evidence also demonstrates that these materials are highly injurious to some nontarget species and threaten other species and biological systems. Elimination of all nonessential uses should be achieved and the period of 2 years is recommended to assure achievement without excessive economic disruption.

Unavoidable residues of these persistent pesticides will continue to occur in the soil, water, air, and food supplies for a period of years despite restriction of usage in the United States. Reasonable methods must be established for the use of as much of the food supply as possible without hazard to human health.

Despite diminution of DDT usage, the Commission urges that research be intensified to gain further understanding of the ecological dynamics and public health implications of this example of a persistent chemical widely distributed in the environment.

It should be recognized that DDT is used in developing nations in the prevention and control of malaria, typhus, and other insect-borne diseases, and in the production of food and fiber. The control of such uses is the responsibility of those nations. They should, however, receive from the United States the full benefit of all available information and assistance on hazards, safe and effective uses of pesticides, and alternative methods of pest control.

Recommendation 4:

Restrict the usage of certain persistent pesticides in the United States to specific essential uses which create no known hazard to human health or to the quality of the environment and which are unanimously approved by the Secretaries of the Departments of Health, Education, and Welfare, Agriculture, and Interior.

Several pesticides other than DDT are persistent and cause or can cause contamination of the environment and damage to various life forms within it. These include aldrin, dieldrin, endrin, heptachlor, chlordane, benzene hexachloride, lindane, and compounds containing arsenic, lead, or mercury. We may anticipate that decreased use of DDT and the above chemicals will result in an increased use of other chemicals such as toxaphene. While there is no evidence that toxaphene undergoes biological magnification, its chemical properties suggest that it should receive close surveillance. Furthermore, the use of organometallic compounds and salts of heavy metals other than arsenic, lead, or mercury should be periodically reviewed.

The uses of persistent compounds should be fully reviewed in light of recent advances in understanding the undesired effects of some pesticides. The acceptable uses should be selected and approved unanimously by the appropriate departments. Such usage should be retricted to essential purposes, limited to the lowest effective dosage required for the production and protection of essential foods and fibers, and replaced by safer alternatives wherever possible.

It is, however, impractical to attempt to eliminate the residues of such pesticides from foods by the application of zero tolerance limits. Modern techniques have greatly increased the sensitivity of the analytical methods available when the zero tolerance concept was advanced. This fact must be recognized in judging the possibilities of hazards and establishing tolerance limits with a sufficient margin of safety to protect human health and welfare.

Recommendation 5:

Minimize human exposure to those pesticides considered to present a potential health hazard to man.

Decisions on restriction of human exposure to pesticides should be made by the Secretary of the Department of Health, Education, and Welfare. In reaching such decisions, consideration must be given to both the adequacy of the evidence of hazard to human health and possible consequences to human welfare that flow from the imposition of restrictions on human exposure to pesticides.

Accordingly, it is of utmost importance that the results of screening tests be scientifically and rationally considered. The correct interpretation of hazards to human health is sometimes extraordinarily difficult. It must involve the transfer of the results of animal experiments to prediction of human effects. In addition, the screening process frequently involves preliminary examination of the effects of massive dosages, possible contamination of test samples, and other factors which affect proper interpretation.

The health and welfare of the public must be effectively protected. However, it is not in the best interest of the public to permit unduly precipitate or excessively restrictive action based only on anxiety.

In recent screening studies in animals employing high dosage levels, several compounds have been judged to be positive for tumor induction. In similar screening studies other pesticides have been judged to be teratogenic. The evidence does not prove that these are injurious to man, but does indicate: (1) A need to reexamine the registered uses of the materials and other relevant data in order to institute prudent steps to minimize human exposure to these chemicals; and (2) to undertake additional appropriate evaluatory research on representative samples of these substances in order to guide future decisions. It is further important to have detailed knowledge of sample composition and purity. These materials are: aldrin; amitrol; aramite; avadex; bis (2-chloroethyl) ether; chlorobenzilate; p,p'-DDT; dieldrin; heptachlor (epoxide); mirex; n-(2-hydroxyethyl)-hydrazine; strobane; captan; carbaryl; the butyl, isopropyl, and isooctyl esters of 2,4-D; folpet; mercurials; PCNB; and 2,4,5-T.

The imposition of restrictions on exposure, particularly from pesticide residues in food and water, should be accompanied by periodic review and adjustment of pesticide residue tolerances. Indiscriminate imposition of zero tolerances may well have disastrous consequences upon the supply of essential food and threaten the welfare of the entire Nation. Stepwise lowering of pesticide tolerance may in some cases be an effective and flexible instrument with which to execute policy.

Currently our national resources of funds, manpower, and facilities will not permit the concurrent testing of all pesticidal compounds. Priorities for testing must be established. Effective national implementation of this policy will require continuing development and evaluaton of scientific information concerning the hazards of pesticides to human health. Additional chemicals are being or should be investigated and evaluated for potential hazards to human health, as resources permit.

Recommendation 6:

Create a pesticide advisory committee in the Department of Health, Education, and Welfare to evaluate information on the hazards of pesticides to human health and environmental quality and to advise the Secretary on related matters.

The Secretary of the Department of Health, Education, and Welfare is obligated to protect and enhance human health and welfare. In relation to pesticides, this requires that he draw upon a wide range of expert opinion and guidance. Excellent competence in some areas exists within the staff of the Department, but the advisory services of a group drawn from the professional, industrial and academic specialists in related fields can provide unique and essential services. A Pesticide Advisory Committee should be created and should include experts on human health and welfare, on environmental and agricultural sciences, and from appropriate economic and industrial areas of knowledge and experience.

In assisting the Secretary, the Pesticide Advisory Committee would:

- Intrepret new information from scientific sources and from increased national experience with pesticides.

- Assess the potential hazards of specific pesticides, based on consideration of persistence of residues, possible distribution and magnification in biological systems, and potential hazards to human health and welfare.
- Recommend improvements in administrative procedures relating to pesticides; areas requiring intensified research attention; adequate programs for monitoring and interpreting pesticide distribution; and, in conjunction with the U.S. Department of Agriculture and the U.S. Department of the Interior, recommend educational and training programs designed to improve usage of pesticides and reduce deleterious effects.
- Evaluate the complex risks and benefit considerations necessary for making responsible judgments on the uses of pesticides, and suggest means for maximizing benefits and minimizing risks.
- Provide advice on suitable standards and tolerances for pesticide content in food, water, and air to protect the public health and the quality of the environment.
- Identify gaps in knowledge and advise on needed research.
- Review and recommend test procedures and protocols to be employed by manufacturers in establishing the safety of pesticides.

The committee should receive the full benefit of the information and professional competence present in the Department of Health, Education, and Welfare, maintain strong liaison with any comparable advisory groups in other Federal agencies, and have free access to specialists and experts throughout the nation.

Recommendation 7:

Develop suitable standards for pesticide content in food, water, and air and other aspects of environmental quality, that: (1) protect the public from undue hazards, and (2) recognize the need for optimal human nutrition and food supply.

In setting tolerances for pesticide residues in or on foods, the Department of Health, Education, and Welfare should be cognizant of the need for optimal human nutrition and food supply. Because widespread environmental contamination by DDT and other persistent pesticides can cause unavoidable residues in many raw foods, the new Pesticide Advisory Committee should examine the problem of tolerances carefully. Total human exposure, actual daily intake, and total body burden of pesticide residues should be minimized whenever feasible, but unavoidable residues should be realistically considered. (See next recommendation).

There is need to abate widespread contamination of the environment in order to reduce unavoidable residues of pesticides in food, water, and air. Of equal importance is the need to take anticipatory regulatory action to prevent future problems caused by other pesticides.

The fact that DDT residues are widespread throughout the environment has lead to unusual difficulties for certain food industries. For example, the fact that DDT is concentrated from contaminated waters into the fat of coho salmon and other fish is not the fault of the fishing industry. DDT contamination of lakes, rivers, and oceans is not susceptible to immediate correction and reduction will require concerted action to prevent DDT entry from various sources. Tolerances for DDT residues in fish should be subjected to immediate review and reflect the relative importance of the food in the diet. Concurrent efforts should be made to apply processing methods capable of reducing the DDT content of fish.

The Pesticide Advisory Committee may wish to consider a graded series of regulatory actions developed in proportion to the extent of environmental contamination or risk thereof, in relation to total human exposure, actual daily intake, and total body burden of pesticide residues. Such a series might be as follows:

Grade I: No significant environmental contamination or risk thereof is judged to exist. Only periodic surveillance and confirmatory evaluation is required.

Grade II: Some environmental contamination or risk thereof is judged to exist. Active surveillance is required, including the assessment of total human body burden of pesticide residues; investigation of their relationships to various sources; evaluation of reductions by variations in food harvesting, processing, and distribution techniques; and routine regulatory controls.

Grade III: Substantial environmental contamination or risk thereof is judged to exist. Restrict total usage with active program of replacing present usage with alternative pesticides, and approve use by permit only.

Grade IV: Widespread or severe environmental contamination or general risk thereof exists. Ban all nonessential uses and remove from the general market. Approve use by permit only.

If indicated by the best available evidence, the above-graded series of regulatory actions would permit immediate classification of a pesticide into grade III or IV, thus requiring approved use by permit only. Anticipatory action can prevent harmful environmental contamination by pesticides and their movement into food, water, and air.

Recommendation 8:

Seek modification of the Delaney clause to permit the Secretary of the Department of Health, Education, and Welfare to determine when evidence of carcinogenesis justifies restrictive action concerning food containing analytically detectable traces of chemicals.

The effect of the Delaney clause[2] is to require the removal from interstate commerce of any food which contains analytically detectable amounts of a food additive shown to be capable of inducing cancer in experimental animals. This requirement would be excessively conservative if applied to foods containing unavoidable trace amounts of pesticides shown to be capable of inducing cancer in experimental animals when given in very high doses. If this clause were to be enforced for pesticide residues, it would outlaw most food of animal origin including all meat, all dairy products (milk, butter, ice cream, cheese, etc.), eggs, fowl, and fish. These foods presently contain and will continue to contain for years, traces of DDT despite any restrictions imposed on pesticides. Removal of these foods would present a far worse hazard to health than uncertain carcinogenic risk of these trace amounts.

Commonly consumed foodstuffs contain detectable amounts of unavoidable naturally occurring constituents which under certain experimental conditions are capable of inducing cancer in experimental animals. Yet, at the usual low level of intake of these constituents they are regarded as presenting an acceptable risk to human health.

Exquisitely sensitive modern analytical techniques which became available since enactment of the Delaney clause permit detection of extremely small traces of chemicals at levels which may be biologically insignificant. Positive response in carcinogenic testing has often been shown to be dose-related, in that the carcinogenic response increases with increasing dose levels of the carcinogen; when the dosage of a carcinogen is minimized, the risk for cancer is also minimized or eliminated.

The existence of such dose responses of carcinogens must be taken into account by evaluating the balance of benefits and risks as is commonly done in assessing any toxic chemical. Ignorance concerning the possible role of environmental chemicals in causation of human cancer reinforces the case for caution in making such judgments. In addition to the complexities of determining a "no effect" level of a weak carcinogen in a given experimental species, the extrapolation of the substance's effects to other species including man is of such intuitive nature that a wide margin of safety must be allowed. Nevertheless, compelling considerations of the increasing need for food may lead to acceptance of an undetectable small risk in order to obtain the benefit of adequate food.

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[2] Federal Food, Drug, and Cosmetic Act, as amended, sec. 409(c)(3)(A).

The recommendation for revision of the Delaney clause is made in order to permit determinations essential to the protection of human health, not to justify irresponsible increases in the exposure of the population to carcinogenic hazards.

Recommendation 9:

Establish a Department of Health, Education, and Welfare clearinghouse for pesticide information and develop pesticide protection teams.

The sources of information on pesticides are exertmely diverse and scattered, including Federal and State agencies, universities, private research centers, and industrial laboratories. The urgent problems of pesticide management require rapid access to scientific information. At the same time, a most serious information gap exists in the absence of reliable sources of data on local activities, progress, and problems throughout the Nation. Therefore, the establishment of a clearinghouse for pesticides is recommended and the organization of pesticide protection teams is strongly urged.

The clearinghouse should:

- Collect and organize information on pesticides and their relationships to human health and the quality of the environment in a modern system for storage, retrieval, and dissemination, and secure evaluations of such data.
- Provide bibliographies, reprints, and summaries upon request from the Secretary of the Department of Health, Education, and Welfare, appropriate Federal and State agencies, research centers and others with a valid need for knowledge.
- Receive continuously information from the pesticide protection teams and provide for its proper summary and distribution, with special attention to dangers or improvements related to methods of pest control.
- Maintain liaison with national and international bodies active in the field of pesticide safety.
- Receive, summarize, and distribute data from pesticide monitoring programs related to human health and welfare.

Pesticide protection teams should be developed from existing local personnel and coordinated with Federal and State personnel and facilities from agriculture, wildlife and public health. They would:

- Augment existing agricultural extension and fish and wildlife efforts relating to pesticides and thereby guide local usage and safeguards.

- Improve local surveillance of pesticide contamination, facilitate monitoring of human tissue residues of pesticides, and investigate usage patterns and episodes of human toxicity.
- Provide a rapid flow of local information based on the above activities, to and from the clearinghouse, especially concerning any emergency related to pesticides.
- Inform the public, users of pesticides, local government and enforcement agencies, and others in the proper and safe uses of pesticides, techniques for disposal, and other matters.
- Stimulate local awareness and constructive concern essential for optimal use of pesticides.

Recommendation 10:

Increase Federal support of research on all methods of pest control, the effects of pesticides on human health and on the ecosystem, and on improved techniques for prediction of human effects.

The scientific talent of the Nation should be mobilized more effectively to resolve the problems associated with the control of pests. This will require increased Federal support of intra- and extramural research and development by all Departments concerned with pesticides.

In order better to assess the toxic effects of pest control agents on nontarget organisms and on human health, research should be expanded relative to the metabolism and degradation of pesticides and their effects on the integrated systems by which organisms derive energy, build protoplasm, and reproduce. Additional studies on teratogenesis, mutagenesis, and carcinogenesis must be supported. Epidemiologic and pathologic relationships as may exist between pesticides and hematologic, metabolic, neurologic, cardiovascular, and pulmonary diseases, pregnancy losses, and cancer must be studied in appropriate communities and population groups.

The nature and extent of any interactions that may exist between pesticides and other factors in the environment require further elucidation. Improved scientific methods and protocols should be developed to assess dose-response and metabolic phenomena related to the biological effects of pest control chemicals in various species in order to increase the accuracy of extrapolative predictions concerning human effects.

The economic costs of pesticides should be evaluated. This should include the hidden costs to man resulting from the uses of pesticides. Accurate quantitative data on environmental contamination and damage to nontarget species by pesticides should be obtained in order to assess the impact of the total global burden of pesticide residues. The Department of Agriculture undertakes to determine the economic impact of changes in pesticide usage, and such information should be incorporated in this evaluation.

Cooperative Federal and State programs of research, training, and demonstration aimed at the solution of practical pest control problems should be expanded. The U.S. Department of Agriculture and the Department of the Interior should make greater use of cooperative agreements and grant support for these purposes. Such support would lead to:

a. Better evaluation of the benefits of pesticides used for various purposes in the context of alternative methods of pest control, including combinations of pest control methods;
b. Development of less hazardous pest control chemicals with high target specificity and minimal environmental persistence;
c. Comprehension of the nontarget effects of pesticides; and
d. Reduced damage to the environment.

Recommendation 11:

Provide incentives to industry to encourage the development of more specific pest control chemicals.

Incentives should be provided to industry to encourage the development of safer chemicals with high target specificity, minimal environmental persistence, and few, if any, side effects on nontarget species. Developmental costs will be disproportionately high in relation to profits from the lower volume of sales of more specific chemicals which will be used selectively. The high cost of development will discourage investments unless incentives are provided.

In order to encourage joint developmental efforts by Government and industry, consideration should be given to the applicability of the present patent laws and practices. The working life of a patent is in effect shortened by the extended period required to secure approval and registration. Moreover, the assignment of patents to public ownership rather than to licensees reduces the incentive for private enterprise to undertake the financial burden of approval and registration.

Recommendation 12:

Review and consider the adequacy of legislation and regulation designed to:

1. Improve the effectiveness of labeling and instructions to users.
    a. Advertising inconsistent with the label should be prohibited.
    b. An entirely new scheme of denoting relative toxicity should be devised. The average consumer does not understand the progression from caution, to warning, to poison. A need exists for a nonlanguage (graphic or numeri-

cal) representation of oral inhalation and dermal toxicity to enable consumers to select less hazardous materials.

   c. Effective labeling practices and instructions to users require use of common (generic) names for all pesticides, and the conveying of clear directions for and information about proper use, dangers, and first aid. Printing should be readable and multilingual when that is appropriate.
   d. When the chemical is known to be especially hazardous to some type of organism, as toxaphene is for fish, this should be stated.
   e. Instructions should also offer clear directions for safe disposal of the empty container and of any unused material.

2. Extend the present concept of experimental permits as a mechanism to register pesticides initially on a restricted basis to enable close observation, documentation, and reassessment of direct and indirect effects under conditions of practical usage.
3. Improve packaging and transportation practices in order to minimize dangers of spillage and the contamination of vehicles and of other merchandise.
4. Provide for monitoring and control of effluents from plants manufacturing, formulating, and using pesticides.
5. Provide uniform indemnification to parties injured by mistakes in pesticide regulatory actions by Federal and State authorities.

Recommendation 13:

Develop, in consultation with the Council of State Governments, model regulations for the collection and disposal of unused pesticides, used containers, and other pesticide contaminated materials.

The current model pesticide law recommended by the Association of American Pesticide Control officials does not cover the important problems of disposal of surplus pesticides and of used pesticide containers. Regulations to control these important sources of contamination and of accidental poisonings properly belong in State or local codes.

An additional feature that should be included in a model law is registration, possibly by social security number, of all workers employed in manufacturing or applying especially hazardous pesticides. This would facilitate implementation of measures to protect them from hazardous exposures as well as to expedite epidemiological investigation of adverse effects of pesticides on human health.

Recommendation 14:

Increase participation in international cooperative efforts to promote safe and effective usage of pesticides.

DDT is widely distributed throughout the global environment. If present usage patterns continue, or if other persistent pesticides are used in large quantities, the contamination of the environment may increase with time. An international problem exists and it will require international cooperation to solve it.

Government and industry should increase their participation in international cooperative efforts to assist developing nations to promote safe and effective usage of pesticides for disease prevention and the production of essential foods and fibers. The risk-benefit considerations differ somewhat from country to country depending on the particular problems encountered. Both benefits and hazards of using a pesticide must be evaluated carefully in order to determine the appropriateness of use in a given area.

The U.S. Government should assume leadership in studying the inherent health hazards of pesticide usage and cooperate in the training of technical personnel from other countries.

SUMMARIES OF SUBCOMMITTEE REPORTS

USES AND BENEFITS OF PESTICIDES

SUMMARY AND CONCLUSIONS

The production and use of pesticides in the United States is expected to continue to grow at an annual rate of approximately 15 percent. Predictions are that insecticides will more than double in use by 1975 and herbicides will increase at an even more accelerated pace. The foreign use of pesticides will likewise continue to increase with the organochlorine and organophosphorus insecticides continuing to represent a significant part of the foreign market.

The use of DDT in domestic pest control programs is rapidly declining with the major need reported to be associated with cotton production in the Southeastern United States. Although the total production is declining, an increasing quantity is being purchased by AID and UNICEF for foreign malaria programs.

Most other persistent pesticides have continued to decline in use since 1957, a trend that will continue with the remaining uses being primarily nonagricultural. The shift to nonpersistent pesticides will continue at an accelerated rate, however, there will be a continued need for use of persistent materials for the control of selected pest problems.

Although imaginative and exciting research is in progress, noninsecticidal control techniques are not likely to have a significant impact on the use of insecticides in the foreseeable future. There is evidence of an increased appreciation for the use of integrated control in the management of pest populations with less persistent and more selective insecticides playing an important part.

There is a serious lack of information available on pesticide use patterns, particularly as they relate to nonagricultural uses. Likewise, available data are usually not obtainable for a proper evaluation of the economic implications of pesticide use. The United States activity in international pest control programs is complicated by the magnitude of involvement and the complexity of diplomatic and agency responsibilities. There are many factors that are influencing the changing use patterns of pesticides. In addition to new pest infestations, resistance to selected pesticides, alterations in the economics of crop production, and changing agricultural and social patterns, the impact of public opinion is having a growing influence on the use of pesticides. The increased concern for new legislation and regulation of the manufacture, sale, and use of pesticides must not be so structured as to destroy the incentive for development of new pesticides more compatible with other desirable environmental qualities.

CONTAMINATION

Summary and Conclusions

The subgroup on contamination has examined the present status of knowledge on the dissemination of pesticides into the environment, the mechanisms and rates at which they accumulate in various elements of the environment, and methods by which pesticides might be controlled so that their presence in the environment would pose a minimal hazard to society consistent with the benefits to be obtained from their use.

The subgroup has examined: a) The air route by which pesticides are applied and distributed in the biosphere; b) the water route; c) the food route; d) soil contamination; e) household uses of pesticides; f) occupational exposures resulting from the manufacture and application of pesticides, and accidents that may occur in their use; g) alternatives to the use of persistent pesticides; h) the monitoring of pesticides in the environment; i) systems analysis of pesticides in the environment.

Much contamination and damage results from the indiscriminate, uncontrolled, unmonitored and excessive use of pesticides, often in situations where properly supervised application of pesticides would confine them to target areas and organisms and at the concentrations necessary for their beneficial use without damage to the environment. Research investigations, demonstrations, and monitored operations reveal that the careful application of many of the pesticides and the use of techniques presently available and being developed can be expected to reduce contamination of the environment to a small fraction of the current level without reducing effective control of the target organisms.

The present piecemeal involvement of various Federal agencies in pesticide control requires more than the existing type of coordination.

As human health and welfare are the values of prime concern, the DHEW should provide a lead in the establishment of a mechanism for administering pesticide control programs.

Ad hoc studies of pesticides in the environment are not adequate to assess the inputs of pesticides to the biosphere, their degradation, translocation, movement and rates of accumulation. Monitoring is conducted by a large number of agencies, but in each instance the monitoring is related to a specific mission of the agency. Therefore, a single agency should take the initiative to insure the effective monitoring of the total environment, and the filling of gaps in data such as for oceans and ground water, as they are identified. A continuous systems analysis of pesticides in the environment needs to be conducted.

Aerial spraying should be confined to specific conditions of lapse and wind that will preclude drift. Regulations to limit aerial application to specific weather conditions would be helpful in providing guidance for regulatory programs. Increased engineering development effort is needed for the design of equipment for, and the adaptation of helicopters to the aerial spraying of pesticides.

The use of low volume concentrated sprays should be encouraged. Since this technique, if it is not properly controlled, can be more hazardous to workers, effective regulations must precede its increased use.

Increased information is needed on the degree of exposure of the general population to pesticides used for household, lawn, and gardening purposes. More effective means for regulation and control of pesticide use by the general public should be instituted, possibly by licensing of distribution outlets.

The use of lindane and similarly toxic materials which act by evaporation must be discontinued where humans or foods are subject to exposure, such as in homes, restaurants, and schools.

There is a vastly increased need for the education of the general public in the management of pesticides and in the training of professional applicators. Public communications media, schools and universities all have important roles to play.

Labeling regulations must also be improved. Print should be enlarged and language should be made intelligible for the lay public. A need exists for nonlanguage, internationally intelligible insignia or markings that will advise the user of the degree of toxicity and persistance of the product, its method of application, and the target organisms.

More vigorous effort is needed to replace the persistent, toxic, and broad-spectrum pesticides with chemicals that are less persistent and more specific. Certain of the less-persistent pesticides, however, may be more toxic to humans and therefore effective regulation of their application is required to insure against injury to personnel.

Integrated control techniques for the control of select pests promises to effect a reduced usage of pesticides. Such alternative techniques should be more widely applied.

Licensing of commercial pesticide applicators, as well as other large-scale applicators of hazardous materials should be required.

Analytical methods, although extremely good, require further development. Need exists for standardizing or referencing additional techniques, even on an international basis. There is need for both less sophisticated techniques for field use as well as for automated techniques for wide-scale monitoring.

Standards for selected pesticides should be included in the Public Health Service "Drinking Water Standards". Although guidelines and criteria for some pesticides have been delineated, they have never been officially established.

Prior to application of pesticides to waters for the control of weeds, snails, mosquitoes, and in other aquatic uses, a careful analysis should be made of the proposed pesticide characteristics with respect to the uses of the target area. Special concern is indicated where domestic water supply is involved, or where food-chain concentration may occur.

Steps should be taken to prevent the simultaneous shipment of pesticides and foodstuffs within the same vehicle. Comprehensive regulations for pesticide transportation are required.

Safe methods of disposal of pesticides, their wastes, and containers are needed to prevent the contaimination of the environment and to protect individuals from contamination and accidents.

Intensified research and development is needed in the following areas, among others:

a. Prediction of the micrometeorological conditions suitable for aerial spraying.

b. Application of systems analysis to the pesticide-enviroment problem.

c. Pesticide chemodynamics, with emphasis on reservoirs of storage.

d. More intensive development of less-persistent pesticides with narrow spectra of toxicity.

e. Continuing development of spray devices with narrow spectra of droplet sizes.

f. Continuing development of alternatives to chemical control of pests.

g. Creation of more suitable materials for pesticide packaging and containers to facilitate safe transfer, handling use, and disposal.

h. Treatment processes for the elimination of pesticides from domestic water supplies as well as from wastewaters.

i. Immediate studies of the effects of pesticide residues on algal photosynthetic activity.

EFFECTS ON NONTARGET ORGANISMS OTHER THAN MAN

SUMMARY AND CONCLUSIONS

Man is an integral part of the living system, which includes about 200,000 species in the United States. Most of these are considered to be essential to the well-being of man. Pesticides are now affecting individuals, populations, and communities of natural organisms. Some, especially the persistent insecticidal chemicals such as DDT, have reduced the reproduction and survival of nontarget species.

Pesticides are dispersed via air, water, and the movements of organisms. The most significant concentrations are found in and near the areas of intensive use, but traces have been found in the Antarctic and other areas far from application. Pesticides have reduced the populations of several wild species. Both extensive field data and the results of excellent controlled experiments demonstrate that certain birds, fishes, and insects are especially vulnerable. There are suggestions that pesticides in the environment may adversely affect processes as fundamental to the biosphere as photosynthesis in the oceans.

However, the scarcity of information concerning the influences of pesticides on natural populations prevents adequate assessment of their total effects. Less than 1 percent of the species in the United States have been studied in this connection, and very few of these have been subjected to adequate observation. Present methods and programs for determining the influences of pesticides on nontarget organisms are inadequate. Little data exists on the distribution, location, and impact of various pest control chemicals in the natural living systems of the world.

The general nature of the effects of pesticides on nontarget species populations and communities can now be suggested. Although there is usually greater similarity of reaction between closely related species, each species reacts differently to specific pesticides. DDT, for example, causes egg shell thinning in ducks and falcons, but not in pheasants and quail. Pesticides from the air, water, and soil may be concentrated in the bodies of organisms. The concentrating effect is frequently enhanced as one species feeds on another and passes the pesticide from one link to another in the food chain. Hence, predators like some birds and fish may be exposed to levels several thousand times the concentration in the physical environment. Some nontarget organisms can, under highly selective pressure from pesticides, evolve resistance to them. The surviving resistant individuals may pass extremely high concentrations to their predators. In communities exposed to pesticides, the total number of species is usually reduced and the stability of populations within the community is upset. Often, beneficial species are unintentionally eliminated. Such a reduction in the number of species is frequently followed by outbreaks or population explosions in some of the surviving species, usually those in the lower parts of the food chain. When a vital link low in the food chain is eliminated, many predators and parasites higher in the food chain are often also destroyed.

The Committee has reached the following conclusions:

1. Adequate methods should be developed and utilized for evaluation of the hidden costs of the uses of pesticides.

> Such evaluation is essential as part of the development of useful estimates of all of the benefits and costs to society. Some partial estimates of the direct benefits are available and useful. Adequate data are not available on such indirect costs as losses of useful fish and wildlife, damage to other species, and any esthetic effects. These are required to guide rational decisions on the proper uses and control of pesticides so that the net gains will be as great as possible while the net losses are minimal.

2. Persistent chlorinated hydrocarbons which have a broad spectrum of biological effects, including DDT, DDD, aldrin, chlordane, dieldrin, endrin, heptachlor, and toxaphene, should be progressively removed from general use over the next 2 years.

> These pesticides are causing serious damage to certain birds, fish, and other nontarget species among world populations. Some of these species are useful to man for food or recreation, some are essential to the biological systems of which he is a part, and some merit special protection because they are already endangered.
>
> These pesticides have value in specific circumstances, and we suggest that they be used only under license and with special permits. The system for assuring this careful use should be established as the unrestricted use of these materials is phased out over the 2-year period.

3. The release of biocidal materials into the environment should be drastically reduced.

In addition to restriction of the use of hazardous pesticides, many techniques can be applied which will minimize the release of pest control chemicals. In industry, improved chemical and engineering processes could reduce the quantity of contaminated wash water; more effective methods can be developed for disposal of unused stocks and residues of pesticides; and improved surveillance of effluents would be desirable. For home use, improved materials and methods of application can be created and employed with greater discretion on the part of the individuals involved. For large-scale applications, conversion to integrated methods of pest control, care in the selection and application of specific chemicals, and preference for short-lived pesticides would reduce release to the environment.

These efforts, combined with increased research and education, would slowly but effectively reduce the damage to nontarget species.

4. The U. S. Department of Health, Education, and Welfare or another Federal agency should negotiate a contract with a suitable national professional organization to develop a system, complete with standards of training, testing, and enforcement, for the effective restriction of use of selected pesticides known to be especially hazardous to man or to elements of the environment.

To achieve an adequate and prompt further reduction in the use of certain pesticides and still permit their use where no adequate substitute is acceptable, there must be a system of regulation based upon State or local authority but using uniform national standards. This system should provide for use of the selected pesticides only by or under the immediate supervision of a licensed operator meeting certain standards of training, competence, and ethics.

5. Educational efforts relating to the proper and improper usages of pesticides should be improved and expanded.

The most important element in the wise use of pesticides is the individual person who selects the chemical to be used and decides upon the methods of application. Suggestions have been provided elsewhere for the proper training of all large-scale applicators. It is equally important that homeowners, gardeners, students, legislators, civic officials, and others receive adequate and correct information and develop proper attitudes. Such education could contribute greatly to wise use of pesticides, and also to rational response to governmental efforts to protect public health and welfare while gaining as much advantage as possible from pest control methods.

6. All pertinent Federal and State agencies should review and improve policies and practices of pesticide use.

The beneficial uses of pesticides have been accompanied by a wide variety of policies and practices which have sometimes been wasteful, unnecessarily destructive, or ineffective. We offer the following suggestions to be included among the guidelines for wise use of pesticides:

a. Pesticides should be applied only when there is evidence that pest densities will reach a significant damage threshold.

b. Effective pest control does not usually require eradication of the pest species, and should be directed toward optimal management of pest densities.

c. Support for research and demonstrations should be provided to projects based on the systems approach to pest management and control.

d. Diversity of species is biologically desirable since it contributes to the stability and efficiency of life systems.

e. No species should be eradicated except as a carefully selected pest and when compensating human gains are ecologically sound and clearly established.

f. Special care must be taken to prevent any damage to the species and mechanisms which are of fundamental importance to biological systems. For example, oceanic phytoplankton produces most of the oxygen necessary for the earth's biological system.

g. Requirements for food quality should not be so high as to require excessive use of pesticides. Customer preference, and regulatory requirements, for unblemished fruit and vegetables and the complete absence of insect parts have encouraged heavy use of pesticides.

h. New pesticides should be given interim approval which permits contained use in limited but typical circumstances prior to general approval. The pattern of careful progressive risks would encourage new developments without endangering the public interest.

i. Effective incentives should be established to encourage the development of improved pest control techniques. The cost of entering a new product or testing a different control technique is high. Since effects on the national welfare are involved, proper governmental encouragement of private industrial efforts may be appropriate.

7. Registration requirements should be strengthened and redesigned to permit initial provisional approval, then general use approval, and to require periodic review and re-registration of materials.

Registration of pesticides offers the most important opportunity for estimating potential benefits and costs in advance of wide usage. In addition to present registration application information, useful estimates should be provided of the persistence of the pesticide, on the breadth of its biological impact, and on its fate. These will disclose the nature and possible magnitude of the nontarget effects. If approval is appropriate, we suggest that it be for a short-term period and for use under defined circumstances where risks are confined, and that general use be considered after such field experience. Since some of the significant effects in nontarget species are subtle, sublethal, and difficult to detect, we recommend that all pesticides be subject to periodic review and approval.

8. All commercial applications and other large-scale applications of pesticides should be performed under the supervision of competent trained persons.

The complex responsibilities of pesticide application involve both achievement of the greatest possible benefit and maximum prevention of damage. These require considerable knowledge of the management of crops, the biology of desirable and undesirable species, the effects of weather, and the effects of biocide in the ecosystems. They also require application of professional judgment and use of professional standards of conduct and responsibility. We suggest that all such applicators should be properly trained, required to demonstrate their competence, and awarded evidence of their ability. Incentives in the forms of salary and recognition will be needed to encourage such professional training.

Training programs for pest management specialists of all types, including applicators, should include the concepts of systems approaches to pest control and emphasize the relationships between pest management activities and the total biological community affected.

Since new information is emerging rapidly in pest management, refresher courses for county agricultural agents, applicators and others involved in the uses of pesticides and other control techniques would be of special value.

9. The production of additional information and comprehension should be encouraged and supported on many aspects of pesticide use and effects.

Experience with pesticides has revealed many serious gaps in available knowledge. Research is urgently needed on many general and specific problems. The following problems are all related to nontarget effects of pesticides, and many of them are also pertinent to other areas of pesticide use, to successful management of animals and plants, and to fundamental science.

a. What are the acute effects of the common pesticides when used on the many species of wildlife and other organisms which may be exposed to them?

b. What are the effects of indirect and chronic exposure?

c. What is the nature and magnitude of the effects of insecticides on beneficial insects and other species?

d. What are the normal patterns and variations in natural biotic communities, as baselines for understanding future pesticide pollution effects?

e. What mechanisms exert natural control on various pest populations?

f. How can we best estimate pest populations and predict their trends?

g. What are the full potentials and realistic limitations of the pest control methods which are suggested as alternatives to chemical pesticides, including predators, parasites, pathogens, cultural control, sterilization, attractants, repellants, genetic manipulation, and integrated approaches?

h. What improvements are possible for pesticide packaging and disposal (including degradable containers) to minimize threats to nontarget species?

10. A vigorous specific program should be created to bring the 100 most serious insect pest species of the United States under optimal control.

These require about 80 percent of the insecticides now in use. Dramatic focusing of attention on the "100 worst" could lead to rapid improvement in the species-specific insecticides, biological control methods, or integrated control programs.

11. The responsibilities of the several Federal agencies involved in pesticide regulation and control must be more clearly defined and certain specific activities should be improved or initiated by appropriate agencies.

Procedures and patterns for the regulation and control of pesticide use have emerged during the last 30 years in response to changes in law, evolving practices in agriculture, production of new chemical materials, changing public concern with health effects and nontarget damage, emerging scientific comprehension of benefits and costs, and other unstructured events. Both benefits and costs are now so large as to merit the national allocation of responsibilities. We suggest careful review and reassignment, by law if necessary, of the proper role of—

a. The Department of Interior, charged with protection and enhancement of nonagricultural resources and with water quality control.

b. The Department of Agriculture, charged with assisting in the maximum production of food, fibers, and other culturable crops in ways which are not detrimental to other interests.

c. The Department of Health, Education, and Welfare, charged with protection and improvement of human health and welfare.

d. The National Science Foundation, responsible for improved comprehension of fundamental processes and assisting in their application for human benefit.

e. The Environmental Quality Council, Federal Committee on Pest Control, and other coordinating agencies.

Other agencies are, of course, involved as users of pesticides and in other functions. Those listed above, however, appear to comprise the areas of primary attention. In addition to present programs and activities related to pesticides, we suggest the following services for new or additional emphasis:

a. A taxonomic and identification service should be established to provide increased knowledge and reference standards for biological investigations related to all fields of pest control.

b. Broader monitoring should be undertaken of the types and quantities of pesticide transmitted by various means and reaching nontarget species. Bioaccumulators like oysters and other molluscs can be unusually useful as indicators, and the levels of concentrations in predatory species are of special importance.

c. Early indications of undesirable effects must be detected effectively and followed by appropriate action. When the early warning system suggests a potential pollution hazard in the environment, the acquisition of additional pertinent information by the scientific community should be supported.

d. Multidisciplinary investigations of alternative control techniques should be carried out whenever present control methods are shown to contain potential hazards.

e. A single agency should assume the responsibility for assimilating information on the effects of pesticides on nontar-

get species and transmitting it to appropriate regulatory and educational centers.

f. Measurable predictors of potential hazards from pesticide use should be agreed upon and might be made the basis of a handicap tax to be applied to each pesticide in proportion to its pollution hazard.

EFFECTS OF PESTICIDES ON MAN

SUMMARY AND CONCLUSIONS

The scope of this report is intended to encompass the present state of knowledge concerning the nature, extent and consequences of human exposure to pesticides. Data relating to exposure of experimental animals have been reviewed only insofar as they contribute to our understanding of phenomena encountered in man or provide knowledge in areas where human data are meager or totally lacking.

No human activity is entirely without risk and this maxim holds for pesticide usage in the human environment just as it does for all other exposure to chemicals. There are formidable inherent difficulties in fully evaluating the risks to human health consequent upon the use of pesticides. In part, these difficulties stem from the complex nature of the problems involved, the fact that many facets of these problems have been recognized only recently, and the general backwardness in this area of research in *man*, as distinct from work in laboratory animals. Above all, one must not lose sight of the large number of human variables—such as age, sex, race, socio-economic status, diet, state of health—all of which can conceivably, or actually do, profoundly affect human response to pesticides. As yet, little is known about the effects of these variables in practice. Finally, one must realize that the components of the total environment of man interact in various subtle ways, so that the long-term effects of low-level exposure to one pesticide are greatly influenced by universal concomitant exposure to other pesticides as well as to chemicals such as those in air, water, food and drugs. While all scientists engaged in this field desire simple clear-cut answers to the questions posed by human exposure to pesticides, the complexity of the human environmental situation seldom allows such answers to be obtained. Attempts to extrapolate from the results of animal experiments to man are also beset with pitfalls. Hence, the greatest care needs to be exercised in drawing conclusions regarding cause-and-effect relationships in human pesticide exposure.

The available evidence concerning such human exposure to pesticides derives from three main sources: planned and controlled administration of pesticides to human subjects; case reports of episodes of accidental or other acute poisoning; and epidemiological studies, which in turn comprise surveys of occupationally-exposed groups (in accordance with a variety of retrospective and prospective approaches), and studies of the general population.

Indices of exposure of human beings to pesticides constitute a vital link in the chain of evidence that must be forged in order to reveal, interpret, and maintain effective surveillance of, pesticide exposures. Hitherto, the view that exposure of the general population was predominantly associated with the presence of pesticide residues in food has been reflected in the efficient monitoring of total diet samples and individual foods, but only sporadic attention to other sources of exposure. It is now evident that much can be learned by monitoring the end-product of human exposure in the form of pesticide levels in body fluids and tissues of people. The information thus obtained is quite distinct from, and at least as valuable as, the data on residues in food; the two types of data complement each other admirably. Provision of information on human levels, in adequately detailed coverage of various groups within the general population is seen as the single most immediate step towards a better understanding and surveillance of total exposure from all sources of pesticides.

Sophistication achieved through the use of modern techniques has made possible the study of absorption, disposition, metabolism and excretion of some pesticides in man. Experience derived from animal studies has provided guidance in directing the appropriate procedures to the investigation of the behavior of pesticides in the human body. To date, the most significant information of this sort relates mainly to two organochlorine pesticide groups, namely DDT and allied compounds as well as the aldrin-dieldrin group. Knowledge of the dynamic aspects of the behavior of these two pesticide groups in the human body is far from complete, but already some important facts have been established. In general, for any given level of pesticide intake, an equilibrium level of pesticide is attained in blood and body fat, despite continuing exposure. The precise concentration at which the plateau is established is directly related to the level of exposure but also to other determining factors. In the case of aldrin-dieldrin, the blood level appears to be a reliable measure of exposure. It appears further, that DDT in blood is directly related to recent exposure, while in contrast DDE in blood is a reflection of long term exposure.

A detailed survey of case reports of incidents involving accidental poisoning by organochlorine pesticides reveals that their general action is to increase the excitability of the nervous system. Some of these compounds also damage the liver. Their capacity to penetrate intact human skin varies from one compound to another; in the case of endrin, for example, percutaneous penetration plays an important part in clinical intoxication. Within the organochlorine group of compounds there is a wide range of potential for acute toxicity: DDT is relatively safe in terms of acute intoxication, while dieldrin and endrin have produced many cases of serious poisoning. Lindane presents a special problem, inasmuch as it has been implicated, largely on the basis of circumstantial evidence, in the causation of hematological disorders. A characteristic of organochlorine poisoning is the difficulty of establishing the correct diagnosis. This is especially true in cases of mild poisoning that result in nonspecific symptoms and signs, since except in the case of dieldrin there are no established criteria for diagnosis on the basis of blood levels. Specific therapeutic measures do not exist.

Inhibition of cholinesterase enzymes by the organophosphate pesticides appears to be the only important manifestation of acute or chronic toxicity produced by this class of compounds. Great variation in acute toxicity from one compound to another characterizes this group, which includes some of the most toxic materials used by man. Cholinesterase inhibition results in a well-defined clinical pattern of intoxication which can be readily diagnosed. Specific therapeutic measures are available and, provided they are pressed with sufficient speed and vigor, are highly effective. Skin penetration by organophosphates may be substantial. In view of the toxic potential of these compounds, protection of workers exposed to them assumes utmost importance. Protective measures should include education, training, proper equipment design, suitable personal protection devices, careful medical surveillance and well-organized facilities ready to treat cases of poisoning with a minimum of delay.

Carbamate pesticides are also cholinesterase inhibitors but, because of rapid in vitro reactivation of the enzyme, measurement of cholinesterase activity is not a reliable guide to exposure. As with organophosphates, the toxic potential of some members of the carbamate group is very great.

Controlled exposure of human volunteers to pesticides under close medical supervision constitutes the most reliable approach to the unequivocal evaluation of long-term effects of low levels of pesticide exposure. The difficulties involved in maintaining such studies have inevitably resulted in very small groups of subjects being exposed for any appreciable length of time. The longest studies on record have lasted less than four years and the results can only reflect the period of study. Consequently, the findings, especially when they are negative, are open to question when taken by themselves. It appears, however, that present levels of exposure to DDT among the general population have not produced any observable adverse effect in controlled studies on volunteers. The same is true of aldrin-dieldrin. These findings acquire greater force when combined with observations on other groups, such as occupationally-exposed persons.

With organophosphate pesticides, the problem of human residues does not arise because these compounds are not stored in body fat. Here the risk is one of acute poisoning. Much accidental poisoning is attributable to public ignorance of the toxicity of these chemicals and neglect of appropriate precautions in their use and storage. In developing countries serious accidents result from storage of pesticides in unlabeled bottles and of food in used pesticide containers. Epidemics of acute poisoning follow spillage of concentrated organophosphates into bulk food or water sources. The hazard to human life is shared by fish and wildlife. Regional pesticide protection teams are suggested as a means of investigating, recording and ultimately preventing accidents of this sort.

Industry has made much progress towards safe handling of pesticides. Nonetheless, a very real occupational hazard exists, and extension of preventive measures should include regular blood testing for evidence of organophosphate exposure. A limit for DDT and other organochlorine pesticides in blood should be established to prevent overexposure.

Pesticide exposure experienced by the population at large is in part the legacy of earlier excessive or injudicious use of persistent pesticides. Residues of these compounds have been, and are still being acquired from all articles of diet and a variety of other environmental sources. This is the major source of public concern. Although a number of persistent pesticides can be identified, attention is centered on DDT, and closely-related compounds, the most ubiquitous and predominant of all pesticide residues in man. The consequences of these prolonged exposures on human health cannot be fully elucidated at present. Evidence from workers who are subject to vastly greater exposure than the public is reassuring but far from complete. Animal experiments clarify certain issues but the results cannot be extrapolated directly to man. On the basis of present knowledge, the only unequivocal consequece of long-term exposure to persistent pesticides, at the levels encountered by the general population, is the acquisition of residues in tissues and body fluids. No reliable study has revealed a causal association between the presence of these residues and human disease.

Despite such reassurance, realization of the paucity of our knowledge in this area flows from increasingly sophisticated studies on human residues of DDT and related compounds. There appears to be marked geographical stratification of DDT residues in our population, the average levels in the cooler isotherms being one-half of those in the warmer climates. None of these observations apply to residues of dieldrin. Such findings cast serious doubt on accepted beliefs that food is the predominant source of DDT residues and that the entire general population has reached equilibrium as regards acquisition of such residues.

Reopening these questions emphasizes the inadequacy of present monitoring of exposure by relying mainly on analysis of food. This aspect was stressed above. It also renders more urgent the need to contain and eventually greatly reduce the extent of human and animal contamination by pesticide residues. Existing knowledge confirms the feasibility of inducing active withdrawal of pesticide residues from the human body but further research to achieve a practical means of attaining this goal is needed.

A survey of the reported effects of pesticides on laboratory animals has furnished information on factors and experimental conditions that could not easily be reproduced in human studies. For example, the influence of diet on pesticide toxicity, and particularly lack of dietary protein, has revealed substantial increases in acute toxicity of some pesticides. In this, as in some other sections of our report reference is made to the capacity of organochlorine pesticides to bring about a great increase in the activity of liver enzymes responsible for the metabolism of foreign compounds. This phenomenon of enzyme induction has been extensively studied in animals and is discussed in detail in the report of the Panel on Interactions. Comparable enzyme induction in the human liver is brought about by many drugs and also by DDT. It is a sad comment on the dearth of knowledge of human physiology to point out that the threshold dose of DDT for induction of metabolizing enzymes in human liver is unknown.

Special sections of the report deal with the possible effects of pesticides in bringing about heritable alterations in the genetic material (mutagenesis), effects on reproduction, including malformations in the fetus or newborn infant (teratogenesis) and increasing the incidence of various forms of cancer (carcinogenesis). The data available relate only to experimental animals or to lower forms of life. At the present time we do not know whether or not such results are applicable to man. While there is no evidence to indicate that pesticides presently in use actually cause carcinogenic or teratogenic effects in man, nevertheless, the fact that some pesticides cause these effects in experimental mammals indicates cause for concern and careful evaluation. It is prudent to minimize human exposure to substances producing these adverse effects in mammals while additional investigations are undertaken to assess the potential of such suspect pesticides for causing adverse effects in man. There is a need to develop standard protocols for safety evaluation that are sufficiently flexible to permit an individual approach to the particular and often unique problems presented by each pesticide. Assurance of safety to man demands special techniques, not only for extrapolation of animal data to man, but also for evaluation of controlled human expsure. Much effort will be required to attain these objectives. Research in these areas should be expanded and imbued with a greater sense of urgency than that manifested before.

The Panel on Interactions has provided a valuable analysis of the manner in which pesticides can interact with one another, and with drugs and other environmental agents, in exercising effects on man and animals. Once again one is struck by the complexity and importance of these interrelationships and by the extent of our ignorance of effects on man.

To sum up, the field of pesticide toxicology exemplifies the absurdity of a situation in which 200 million Americans are undergoing lifelong exposure, yet our knowledge of what is happening to them is at best fragmentary and for the most part indirect and inferential. While there is little ground for forebodings of disaster, there is even less for complacency. The proper study of mankind is man. It is to this study that we should address ourselves without delay.

Part II

Subcommittee and Panel Reports to the Commission on Pesticides

CHAPTER 1

Uses and Benefits of Pesticides

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USES AND BENEFITS OF PESTICIDES

SUMMARY AND CONCLUSIONS

The production and use of pesticides in the United States is expected to continue to grow at an annual rate of approximately 15 percent. Predictions are that insecticides will more than double in use by 1975 and herbicides will increase at an even more accelerated pace. The foreign use of pesticides will likewise continue to increase with the organochlorine and organophosphorous insecticides continuing to represent a significant part of the foreign market.

The use of DDT in domestic pest control programs is rapidly declining with the major need reported to be associated with cotton production in the Southeastern United States. Although the total production is declining, an increasing quantity is being purchased by AID and UNICEF for foreign malaria programs.

Most other persistent pesticides have continued to decline in use since 1957, a trend that will continue with the remaining uses being primarily nonagricultural. The shift to nonpersistent pesticides will continue at an accelerated rate, however, there will be a continued need for use of persistent materials for the control of selected pest problems.

Although imaginative and exciting research is in progress, noninsecticidal control techniques are not likely to have a significant impact on the use of insecticides in the foreseeable future. There is evidence of an increased appreciation for the use of integrated control in the management of pest populations with less persistent and more selective insecticides playing an important part.

There is a serious lack of information available on pesticide use patterns, particularly as they relate to nonagricultural uses. Likewise, available data are usually not obtainable for a proper evaluation of the economic implications of pesticide use. The United States activity in international pest control programs is complicated by the magnitude of involvement and the complexity of diplomatic and agency responsibilities. There are many factors that are influencing the changing use patterns of pesticides. In addition to new pest infestations, resistance to selected pesticides, alterations in the economics of crop production, and changing agricultural and social patterns, the impact of public opinion is having a growing influence on the use of pesticides. The increased concern for new legislation and regulation of the manufacture, sale, and use of pesticides must not be so structured as to destroy the incentive for development of new pesticides more compatible with other desirable environmental qualities.

General History of Pesticides

A pesticide is a chemical used to cause the death of nonhuman organisms considered by man to be "pest"; i.e., inimical to human interests. Rather arbitrarily the following are excluded: pathogenic microorganisms, viruses, bacteria, protozoa generally, endoparasites of man and other animals, and a host of organisms causing special problems such as the marine fouling organisms. Technically materials such as chemotherapeutic agents can be classed as pesticides. However these are regarded as outside the scope of consideration of the commission.

Space does not permit us to trace the history of pesticide usage back to the dawn of recorded history. For example, it has been claimed that Marco Polo brought pyrethrum to Europe from the far east as a wonderous compound of secret origin. Rather, our emphasis will be on the changes in uses and attitudes toward pesticides which occurred quite suddenly in the mid-1940's. The insecticidal properties of DDT were discovered in 1939; it was used in the field, mostly by the military, in the early 1940's, and became commercially available to the public in this and other countries about 1945. Similarly, plant hormones and close analogs were objects of research in the late 1930's, and from these studies came the herbicide 2,4-D which was released commercially almost simultaneously with DDT. From that start a host of new materials has been produced, a major new industry has come into being, and agricultural and public health practices have been revised. Education and research efforts in these areas have not kept pace with developments in industry, agriculture, and public health. As a result, there has developed a public fear and public concern over the usage of these new pesticides. Thus we shall concentrate our attention on this sudden shift in pesticide practices that developed about 1945.

Pesticides before 1945

Perhaps the earliest pesticides to be used were organic materials of natural origin. It is not known when pyrethrum was first used to kill insects nor red squill to kill rats. Preparations of the plant sabadilla have been used as louse powders by South American natives for centuries. As early as 1763 ground tobacco was recommended in France to kill aphids and the active ingredient, nicotine, was discovered in 1809. Plant materials containing rotenone were used as insecticides as early as 1848. Petroleum, kerosene, creosote, and turpentine were also introduced as insecticides in the 18th century. They proved highly toxic to plants as well as insects but the petroleum oils came into wide use as larvicides for mosquitoes. Late in the 19th century highly refined oils with low toxicity to plants were introduced in emulsion form and gained wide usage. A number of other natural organic materials were developed as pesticides and are still in use today although their relative importance has diminished.

Inorganic compounds also came into early use as pesticides. The arsenical, Paris Green, was used against the potato beetle in the Rocky Mountain region as early as 1865. To combat scale insects in California lime sulfur washes and fumigation with hydrogen cyanide (not strictly an inorganic compound) were introduced in 1886. The use of hydrogen cyanide led to what is one of the earliest recorded instances of an insect developing resistance to an insecticidal chemical; by 1916 it was observed that the red scale insect was no longer killed by HCN, and as this resistance spread geographically the use of this control gradually declined. Lead arsenate was introduced as an insecticide against the gypsy moth in Massachusetts in 1892. Sodium arsenite found use both as an insecticide and a weed killer.

Gradually a number of metal salts, including those of copper, zinc, chromium, and thallium, came into pesticide use, as well as some extremely toxic compounds of fluorine and sulfur. Some of the metallic salts such as cryolite (sodium fluoaluminate) and various salts of arsenic, lead, mercury, and selenium are extremely persistent in soils and are removed only by weathering, erosion, or in the bodies of plants that absorb them from the soil (in the case of mercury some escapes into the air by volatilization).

The use of synthetic organic pesticides also began before World War II. Dinitrophenols found very limited use in Germany as early as 1892, and such extremely simple compounds as HCN, carbon disulfide, and methyl bromide were used very early. Unsuccessful attempts to synthesize pyrethrum were begun in the 1920's. Naphthalene and paradichlorobenzene came into use early in the 20th century, and a few thiocyanates and cyclohexylamines were recognized as potential insecticides in the 1930's. The insecticidal properties of DDT were recognized in Switzerland in 1939, and those of benzene hexachloride (BHC) about 1940 in France and England.

With the advent of World War II our supplies of pyrethrum for louse control and red squill for rat control were largely cut off and it was imperative to find substitutes for military purposes. DDT then came into military use and a crash program of screening by the U.S. Fish and Wildlife Service developed the very poisonous compound 1080 (sodium monofluoroacetate) as a rodenticide. The success of DDT in halting a typhus epidemic in Italy in 1943 and 1944 was an unprecedented achievement which heralded the postwar era of unparalleled benefits in the use of pesticides for human health.

Pesticides after 1945

As DDT came into wide use and its extraordinary insecticidal effects were recognized there were predictions that all major insect pests would be eradicated. The dangerous malarial mosquito *Anopheles gambiae* had been eradicated in Brazil in the 1930s using pyrethrum as the principal chemical weapon, and the availability of a low cost synthetic substitute raised hopes for many more such triumphs. However, very soon, limitations to DDT use began to be recognized.

The promises of chemical control were viewed with such optimism that research and agricultural practices often shifted away from techniques such as cultural methods that had formerly been used. Major efforts were devoted to finding new pesticidal chemicals without the limitations that had appeared for DDT. It was found that insects that were resistant to DDT were also often resistant to other related organochlorine compounds thus the search began for insecticidal compounds with distinctly different chemical structure. Then the organophosphates came into use but resistance often rapidly developed. At present we are witnessing an expansion in the use of carbamate insecticides.

Following the appearance of resistance to DDT a tremendous number of new pesticides has appeared. There are now in the United States some 900 active pesticidal chemicals formulated into over 60,000 preparations. These include insecticides, fungicides, herbicides and plant growth regulators. Modern food production programs and modern public health programs are dependent upon the use of these pest control agents.

PESTICIDE PRODUCTION AND USE

The production and use of pesticides in the United States continues to grow in response to the demands of the U.S. users and the increased demand for export. Surveys and reports of government and industrial economists indicate that synthetic organic pesticide production is increasing at approximately an annual rate of 15 percent with an indication of more than $3 billion sales by 1975. This is in contrast to increases of approximately 37 percent for the 5-year period, 1963 to 1967. The total dollar value of all pesticides produced in this country was $440 million in 1964; this has increased to $12 billion in 1969. Herbicide sales, as indicated by U.S. Department of Agriculture surveys, have risen 271 percent since 1963 which represents more than double the rate of increase for all pesticides. Predictions are that insecticides will more than double in use by 1975 to more than $600 million, while herbicide uses will increase to more than double that of insecticides ($1½ billion) during that same period. The value for all herbicides produced increased from $200 million to $800 million in a 5-year period from 1964 to 1969 and is expected to reach $1,350 million by 1974.

Foreign uses for pesticides has continued to expand as indicated by U.S. exports, as well as by surveys of European agricultural chemicals. U.S. exports in 1967 were approximately $196 million. Insecticides were responsible for approximately 60 percent of the international movement. Approximately 45 percent of these exports were represented by the organophosphorous and organochlorine insecticides which were about equally divided. In 1967, there was a reduction in DDT exports and an increase in other organochlorine and organophosphorous materials. Sizeable quantities of pesticides continued to be shipped from the United States to eastern European countries in 1967, with the largest share going to the Soviet Union.

The United States produces from 50 to 75 percent of all pesticides manufactured in the world. However, it is predicted that the percentage of the U.S. contribution is likely to be reduced as other countries develop capacities or increase existing capacities to make these chemicals. It is likewise predicted that insecticides and fungicides will continue to dominate the international market for some considerable period of time.

Insecticides

Although the rate of growth for insecticides is not as spectacular as that for herbicides, manufacturers' sales of synthetic organic insecticides in 1967 reached 301 million, which was 10 percent above the previous year and represented 38 percent of the total share of the pesticide sales. In 1967 the United States exported $150 million worth of insecticides which was an increase of about 14 percent over the previous year. These increases resulted largely from exports of technical organochlorine and organophosphate technical materials.

A 1964 survey by the U.S. Department of Agriculture indicated that U.S. farmers used approximately 2 million pounds each of 12 different insecticides, which accounted for 85 percent of the total volume. Toxaphene was used in largest volume, followed closely by DDT; these two made up 46 percent of total pesticides used in 1964. The same survey indicates that farmers applied two-thirds of the total quantity of all insecticides used on farms on three crops: Cotton, corn, and apples. The cotton market accounted for more than half of the total including about 80 percent of the methyl parathion, 86 percent of the endrin, 70 percent of the DDT, and 69 percent of the toxaphene. The

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corn market accounted for 10 percent of the total, including 96 percent of the aldrin and 84 percent of the heptachlor. Latest data made available by the USDA Economic Research Service from their 1966 pesticide-use survey indicates that farm use of pesticides in 1966 (in terms of active ingredients) was up about 10 percent over 1964. Overall, insecticide and fungicides use (exclusive of sulfur) remained about the same as in 1964 even though the use of insecticides on cotton was down because of the large reduction in acreage. However, herbicide use was up more than 33 percent. Leading products among the insecticides continue to be toxaphene, DDT, and aldrin, accounting for over half of all insecticides used by farmers in 1966. Shifts in ingredient usage among insecticides from 1964 to 1966 showed a slight decrease in the use of chlorinated hydrocarbons and an increase in the use of organophosphorous compounds. The latest production figures (1967) indicate that the organochlorines continue to make up approximately one-half of the U.S. production and of these approximately 50 percent is DDT.

Persistent insecticide use patterns

DDT.—U.S. DDT production during 1967 was 103 million pounds, down approximately 27 percent from 1966. Exports during 1967 were 82 million pounds, down approximately 10 percent. Over half of all DDT exports were in the form of 75 percent wettable powder used primarily for mosquito control. In 1967 five countries: India, Thailand, Brazil, Nepal, and Mexico, received over two-thirds of the export tonnage of this formulation. Economic Research Service surveys indicate that the U.S. output of DDT is approximately 40 percent less than the peaks reached in 1960 to 1963. Exports are claiming an increased share, approximately 70 percent of this production. Domestic uses were reduced nearly 50 percent between 1958 and 1966. DDT has been replaced by less persistent pesticides in many States. Of special significance has been the reduction in its use for large-scale forest insect control programs, mosquito larvicide and in some instances, mosquito adulticide programs, as well as many agricultural uses. The use of DDT on agricultural crops ranges from the more than 200 entries in the Federal recommendations to emergency uses only in certain States.

Large amounts of DDT are used in this country for the production of food and fiber, for control of mosquitoes and bats and for other limited purposes. Substitute products are usually more expensive and sometimes less effective. Many of the substitutes have acute toxicities representing greater degrees of hazards to the user than DDT. Other substitutes have a lower mammalian toxicity, but present a much greater hazard to pollinating insects. Still others are more toxic to fish and wildlife.

It is not possible to summarize the advantages and disadvantages of substitute chemicals that are presently available since there are no general substitutes. Several available partial substitutes have a variety of disadvantages including increased mammalian toxicity, lack of tolerances on food commodities, decrease in insecticidal value, high cost, increased toxicity to bees and pollinating insects, and increased toxicity to fish and wildlife. The biological impact of large-scale use of many substitute chemicals is as yet unknown.

The usefulness of noninsecticidal control techniques has been demonstrated on a laboratory scale for several of the major economic insects. The utilization of the sterile male technique and attractants plus pesticides have been used for the control of the screw worm in cattle and of certain fruit flies, both in the United States and on certain Pacific Islands.

Although the total production of DDT is declining, an increasing quantity of this compound is being purchased by AID and UNICEF for foreign malaria programs. It is estimated that more than half of the total U.S. production of DDT is exported by AID and UNICEF for malaria eradication. It is evident from the production data that the use of DDT in domestic pest control programs is declining and this appears likely to continue.

It is reported by well informed scientists that as far as insect vectors of disease are concerned there are none known which are normally susceptible to DDT that cannot be controlled with a substitute. However, stopping the production of DDT in this country would be a very serious blow to foreign malaria eradication programs, now being supported largely by AID. These are normally under the actual supervision of WHO teams. AID records over the past several years indicate shipments of DDT have varied but the trends are slightly downward. The consensus of AID personnel is that an abrupt withdrawal of other organochlorine compounds would create immediate and critical problems for growers throughout much of the developing world, and could have a deleterious effect on world food production and protection of public health. A gradual withdrawal, slow enough to permit substitution and demonstration of organophosphate, carbamate, and other chemicals, would minimize the impact of such action. Such a change would require a vast educational program. Reports from authorities interviewed indicated that it was likely that malaria programs would gradually be discontinued if they were forced to use substitutes for DDT.

The persistence of DDT is the essential characteristic that makes it effective as a malarial eradication tool. A malarial victim may remain infective for many months to mosquitoes that feed upon him. To interrupt malaria transmission, constant protection against bites from infected vectors must be provided for several years. The maintenance of an insecticidal residue on interior surfaces of homes provides this type of protection. The magnitude of the areas to be covered and the inaccessibility of remote rural areas in underdeveloping countries makes frequent spraying of houses impractical and expensive. With DDT one or two treatments annually are usually sufficient. The application of DDT to the interior surface of houses results in a minimum contamination of the outdoor environment. A recent WHO release summarizes the status as follows: "In considering the pesticide problem, we must not forget the enormous benefits insecticides have brought to humanity. DDT has been instrumental in controlling some of the most important vector-borne diseases of man. The concept of malaria eradication rests completely on its continued use. The record of safety of DDT to man has been outstanding during the past 20 years and its low cost makes it irreplaceable in public health at the present time. Limitations on its use would give rise to greater problems in the majority of the developing countries."

It has also been suggested that banning the use of DDT in this country and at the same time sending it overseas for malaria programs would be looked on with disfavor by recipient countries.

Recent reports by the World Health Organization indicate that the control of many of the most important vectors of human diseases is still entirely dependent on insecticides and no effective or economically feasible alternatives are available. There has been some reduction in the use of DDT in vector control programs partly as the result of progress in malaria eradication and partly due to the development of insect resistance. It is very difficult, if not impossible, to obtain an accurate figure on the amount of Federal money spent on the chemical control of vectors domestically or in foreign countries. In the past 10 years it is well over a half-billion dollars. AID and its predecessors have spent at least this much on foreign malaria programs since they were initiated in the early 1950's. If the crisis with respect to the use of pesticide chemicals for vector control is to be overcome, a large increase in funds for research is mandatory. A substantial investment will be necessary to provide a solution to the problems involved in controlling insect vectors without the use of presently available chemicals. Although DDT is still involved in some of the international food production programs sponsored by U.S. agencies, there is a feeling that a withdrawal or systematic reduction of DDT would have a minimum effect.

There is a principle of international diplomacy which recognizes that a free and independent nation has a right to make its own choice without dictation by any other power. For example, pesticides shipped to a foreign purchaser on his own specifications are exempt from the requirements of the Federal Insecticide, Fungicide, and Rodenticide Act of 1947, as amended. Accordingly, information on pesticide usage must be volunteered by the foreign user or his government rather than demanded by AID as a basis for authorizing loans under which pesticides are to be purchased. All pesticide purchase requests handled under AID or comparable funds should be funneled through an appropriate scientific staff. A followup of the use patterns and effectiveness of the material shipped should be an important prerequisite to any fund release or shipment. Such reports should include a summary of efficacy, reports of accidents to humans or other animals, as well as any adverse effects on wildlife. Such input will require a new philosophy as well as substantial reorganization and increased support for proper handling of future pesticide shipments to foreign countries.

Methoxychlor.—In comparison with DDT, methoxychlor production and usage are at much lower levels. Use levels have been quite stable over the past 15 to 20 years, although its pattern of use has varied and changed somewhat over this period.

Currently, about 75 percent of the methoxychlor sold is used for fly control on cattle and in farm buildings, with the remainder divided between crops, control of elmbark beetles (Dutch Elm disease), grain bin treatment, home garden and household insecticides. The largest recent shift has been in crop use; from primarily fruits and vegetables in earlier years, to forage crops particularly alfalfa weevil control.

Restriction in use of the "persistent" insecticides should have only minor effects on use of methoxychlor: (1) Methoxychlor is not very effective against a number of the pests controlled by these compounds—for example, soil insects such as corn rootworm, wireworms, etc., (2) Other compounds (such as various organophosphates or carbamate insecticides) are available and registered for many uses, and would probably be used more extensively, if the persistent insecticides were banned.

A moderate increase (5–10 percent) in methoxychlor usage is projected in the uses for which methoxychlor already is accepted (cattle, farm buildings, etc.) plus possible increases for area control of mosquitoes, blackflies, etc.

Aldrin.—This compound has been an effective and extensively used soil insecticide. Roughly one-half of the U.S. corn acreage treated with soil insecticide last year was treated with aldrin. Particular insects of economic importance that were controlled are ants, cutworms, wireworms, flea beetles, Japanese beetle grubs, seed corn beetles, seed corn maggots, European chafer grubs, white grubs, corn bill bugs, sugarcane beetles, webworms, white fringe beetle grubs, crickets, and corn rootworm larvae. In areas of rootworm resistance to organochlorines narrow spectrum materials are used at rates sufficient to control rootworm. The highest sales for aldrin were in 1966. In 1968 sales of aldrin had dropped 30 percent and by 1972 the estimates indicate a reduction of 60 percent from the highest sales year.

Dieldrin.—This compound is used widely to control a variety of pests, especially when a long lasting residual effect is desired. These residual uses for dieldrin include its application for termite control, insect control on lawns, turfs, ornamentals and flowers, and at the present time household residual sprays and permanent moth proofing of fabrics. The bulk of the material is used for termite control. In 1968, 81 percent of the aldrin and dieldrin agricultural use was for corn soil insects. Other agricultural uses made up 11 percent and nonagricultural specialty uses including termite control, Government programs and so forth, an additional 8 percent.

The highest sales year for dieldrin was in 1956. Usage has steadily decreased because of resistance in the cotton boll weevil and certain other agricultural pests. This is a 70-percent drop in a 12-year period. Estimates indicate that usage will drop another 10 percent by 1972, practically all remaining uses being for nonagricultural purposes.

Endrin.—The major domestic use for endrin is as a cotton insecticide. The projected use of endrin for this purpose indicates a decrease between 1969 and 1973 as a result of increased insect resistance. Projected use in international areas for endrin indicated a relatively stable use pattern or possibly a slight decrease. All uses of endrin in the United States are on a no-residue basis. Substitute insecticides for endrin are being evaluated in many developing countries. However, economic factors have limited the introduction of substitute materials; for example in India, studies indicate that substitute insecticides for control of rice and cotton insects would increase the cost of treatment 80 to 95 percent.

Heptachlor.—This compound is primarily a soil insecticide. It is anticipated that the heptachlor used in the United States will be primarily for control of the soil insect complex in corn, which will represent between 55 and 75 percent of the domestic use. The second most significant use of heptachlor is in the commercial pest control field. At present this usage for primarily structural termite control represents about 15 percent of the use pattern and is expected to increase to some 34 percent by 1973. In 1960 there was a 50-percent decrease in the use of heptachlor over 1959. This was in response to the Food and Drug Administration's concern for the residues of its metabolite heptachlor epoxide. An important use for heptachlor in early years was the control of alfalfa weevil. A significant reduction in the use of heptachlor occurred between 1963 and 1964 as a result of the residues reported in milk and subsequent removal of heptachlor for this use.

Chlordane.—The agricultural usage of chlordane is primarily as a soil insecticide. It has been especially important in the home, lawn, and garden pest control market. These markets represent its major use, which is estimated at about 70 percent of the total use between the years 1969 through 1973. Approximately 50 percent is estimated to be used in the pest control market for primarily structural termite protection and about 10 percent in the home, lawn and garden use, primarily for turf treatment. About 30 percent is estimated for agricultural usage.

Structural pest control is expected to continue its current rate of expansion of about 10 percent per year. The same expansion can be expected in the industry pesticide purchases, which are now about $40 million per year at the wholesale level. Further use of persistent pesticides in the pest control industry is likely to be influenced by development of resistance in important pests. In the mid-1950's, diazinon replaced chlordane as the dominant insecticide in general pest control, because of resistance. Diazinon resistance has not been a major problem. A carbamate insecticide, such as Baygon, might rise in importance if substantial resistance to diazinon should materialize. Resistance in termites appears to be unlikely as a factor affecting choice of insecticides in the foreseeable future.

The commercial structural pest control industry indicates that it has approximately 18,000 men rendering periodic service. Each technician may treat 10 to 20 premises a day and use a persistent insecticide in almost every one. The principal pests controlled are cockroaches, ants, fleas, ticks and pests of fabric, stored food, and wood products. Practical control requires an insecticidal residue to be available at such time as the pests emerge from concealment, from pupation, or from the egg. Aldrin, chlordane, dieldrin, and heptachlor are the materials primarily used for subterranean termite control. An example of the magnitude of this problem is provided by figures available from the State of Georgia where 46 thousand termite jobs are performed each year. Massachusetts reports nearly a thousand inquiries per year by the public regarding termite control. It has been calculated that three out of five houses in the Midwest can be expected to become infested with termites. This applies to the Detroit, Louisville, Kansas City and Milwaukee areas and assumes a house to have a life expectancy of 35 years. The USDA calculations indicate that the annual cost to U.S. homeowners is approximately half a billion dollars from termite damage. The persistent pesticides indicated above provide 18 to 20 years protection in most instances.

Noninsecticidal control techniques are not likely to have any significant impact on future use of pesticides in household and structural pest control.

The commercial pest control operator is using a small and decreasing amount of DDT. These uses are often involved with the control of bats in structures, house mice where control is not possible with other acceptable materials, and limited uses for ants, cockroaches, garbage pests, scavengers, and ectoparasites.

A large portion of the pesticides used by the Department of Defense is shipped overseas. More goes to Vietnam than to any other country. Relatively little persistent insecticide is used for area control of insects. The Department of Defense does little mosquito-larvae control and malathion or naled are the insecticides most frequently used for adult mosquito control. Mirex is used for fire-ant control on some of the installations in the southern U.S. and 10 percent dieldrin is used on small areas when recommended by the U.S. Department of Agriculture Plant Pest Control Division. Persistent insecticides are used for termite control, some for residual spraying in warehouses and other quarters and for selected insects. The Department of Defense has pest control operations on military properties in the United States and overseas. The Armed Forces Pest Control Board provides coordination and each service conducts programs tailored to resolving a specific problem. None of the Department of Defense budget is commonly allocated for pesticide regulation, and no employees are occupied full time on this matter. The importance of such regulation is recognized and many measures are taken to insure that pesticides are utilized properly. In 1969 $1½ million was allocated for pesticide research by the Department of Defense. The majority of the money was used to support research conducted under contract or grant with only six Department of Defense professional personnel devoting more than 50 percent of their time to pesticide research.

The Department of Defense conducts pest-control programs as required on the 30 million acres of property that it controls in the United States. These programs are regarded as essential for the protection of the buildings and perishable stored products that are required for the Armed Forces. The protection of forest and recreation areas is also of major importance. Pest control to protect the health and welfare of citizens residing on and adjacent to military installations is also important. While price data are not readily available, current costs of the Department of Defense Pest Control Program in the United States is approximately $7.7 million per year.

The organochlorines are used extensively on several of the quarantine programs. These materials are considered to be the most practical and in some cases the only means of treating products as well as processing of cargo areas to prevent the spread of several important insect pests. The organochlorines are the backbone of the present regulatory program for whitefringed beetle, Japanese beetle, European chafer, fire ant, and sweet potato weevil. These scientists responsible for regulatory decisions have indicated that there is no satisfactory substitute for these chemicals to meet the requirements for regulation under provisions of the quarantine laws. There is also the joint agreement with the committee concerning the enforcement of similar quarantine regulations on four domestically quarantined pests—namely, European chafer, Japanese beetle, cereal leaf beetle, and gypsy moth. The Republic of Mexico is also concerned about certain of the U.S. domestic quarantine pests, but it accepts our regulatory programs and certification thereunder for movement in Mexico as it relates to these pests. Some organochlorines are also employed as a basis for allowing movement into the United States of certain products of foreign origin. The principal commodities involved in these regulatory programs are nursery stock, sod, bulbs, corms, and plant crowns, stone and quarry products, industrial supplies, timber products, sweet potatoes, Irish potatoes and transplants. Additional regulatory responsibilities are included with the treatment of areas around processing plants, truck and rail transportation centers, trailer camps, campgrounds, and airports.

Summary of foreign insecticides uses

In general, there is limited information available on the economic impact of pest infestations on foreign agriculture and related pesticide use patterns. However, the summary provided the Commission by Shell Chemical Co. scientists gives an updated appraisal of the international uses of persistent pesticides and is included as appendix A of this report.

Specific data are not available from most foreign countries on past use; however, the Indian report published in 1967 by the Special Committee on Harmful Effects of Pesticides has provided interesting and relevant statistics regarding the use and projected use of insecticides. In India the use of pesticides has increased from the treatment of 10,120 hectares in 1946–47 to about 6.15 million hectares in 1961–62 and has risen to 17.4 million hectares in 1965–66. This latter figure represents about 11.2 percent of the total crop area. The amount of DDT used in plant protection is expected to quadruple from 600 metric tons in 1964–65 to 2,400 metric tons in 1968–69. The amount of aldrin, dieldrin, heptachlor, and chlordane is expected to increase from about

90 metric tons in 1964–65 to 1,050 metric tons in 1968–69, a factor of 12. The report indicates that these increases in the use of organochlorine insecticides are not likely to result in any increased hazard to human beings and domestic animals, but that they may have a significant effect on wildlife. The increase in the use of DDT in plant protection will be accompanied by a planned decrease in its use in public health from 8,426 tons of technical material in 1964–65 to 3,456 metric tons in 1968–69. In 1967, approximately 20,326 metric tons of pesticides, technical grade, were being used, of which 16,262 metric tons were manufactured in India and the remaining imported. Predictions were for the requirement of 77,509 metric tons of technical material for use in 1968–69. Within the period of 1965–66 to 1968–69, it is predicted that the area to be treated with pesticide will increase more than 5½ times and will cover 85 million hectares (54 percent of the total crop area).

Pesticide economics

Prior to 1945 it was common to find statements in the literature estimating the loss to pests of crops and stored commodities in the United States at "10 percent," or "at least 10 percent." One standard textbook of economic entomology (Metcalf and Flint, 1939) attempted to be more thorough and to estimate for individual crops and commodities the loss from insect pests as of 1936 both in percentage of the crops destroyed and in dollar values. The latest revised edition of that textbook gives a revised estimate based on 1957 data showing percentage losses to insects of various crops and stored products which are virtually identical to the 1936 figures. The dollar values of the losses, however, have approximately doubled in most cases, in part reflecting increased prices. In the appendix we have added a final column giving the percentage of the potential crop production lost to insects outside North America.

The economics of pest control is made up of the interrelationships of the benefits, costs, and side effects. That is, the incremental benefits of another unit of control must be equal to or greater than the associated incremental costs. This is not an easy task because in pest control there are many different kinds of pests. Each may be associated with many different damages and there are numerous ways to control each pest. At present, research in this important area has been primarily involved with an attempt to measure in aggregate the effects on farm sales associated with varying levels of pesticide use. The only source of data for this work has been the secondary data from the agricultural census and other data variables from the USDA, such as the pesticide use surveys of 1964 and 1966.

The funds allocated to the Economic Research Service by the Agricultural Appropriations Act of 1965 ($500,000) provided for research on the cost and benefits of alternative methods of controlling plant and animal pests, and the collection of basic data on current uses of pesticides and costs and methods of controlling pests. This research was divided into three phases: (*a*) Biennial surveys of a nationwide sample of farms to obtain data on practices farmers employ to control pests by the use of chemicals, and the costs of these practices; (*b*) analysis of selected alternative methods of pest control with emphasis on comparative costs and returns in selected areas; and (*c*) analysis of the economic implications of alternative methods of pest control both on the farm and for agriculture as a whole.

The economic analysis of the benefits of pesticides is largely an undeveloped area. A full cost-benefit appraisal of pesticides in the American economy or in agriculture has not been made. Gaps in present knowledge of the technical relationship of pesticides in the fields of agriculture, health, and natural resources make such an evaluation difficult.

Available data are not adequate to properly evaluate the economic implications of pesticide use. Estimates of the total volume of production of these chemicals are reasonably accurate, but only aggregate estimates are available to indicate the extent that chemicals are used in agricultural production. Little information is available to indicate the use of the many chemicals in the production of specific crop and livestock commodities. Costs of side effects have not been evaluated for inclusion in the analyses.

Headley, who has pioneered in this area, is quick to point out that there is not a large body of time series data available and, therefore, cross-section studies have been used. These estimates are inadequate in several respects. They are not generated experimentally and they may be measures only of association and not measures of the contribution of pest control with other things constant. In addition, the cross-sectional analysis measures change in farm sales at the prices determined for the year of the cross-section measurement, based on supply and demand in that year. Headley's early results show an incremental contribution of about $4 to $5 per $1 of pesticide expended by a farmer. A later study shows contributions per ounce of technical material by production region. The impact of chemical pesticides is strongest overall in the area south of the Ohio River and west of the Mississippi. The Pacific region, South Plains, and Northeast also devote more production resources to chemical pesticides than does the Corn Belt, North Plains and the mountain regions. Headley points out that part of these differences is due to resource values such as land and labor, but it seems reasonable that they also reflect pest problems, such as biological conditions for pests and the product orientation of agriculture regionally such as cotton, vegetables, tobacco, and fruit, as compared with cereals. Sufficient experimental data are not available nor do they seem to be in the process of development. The general needs appear to be:

1. Functions that relate pest infestation to crops or livestock yields both quantitative and qualitative, and that are representative for products produced, pest infestations, and regions.
2. Functional relationships that relate pest control by the various methods, compounds, cultural pratices, biological control methods, to pest infestation, not only for average seasons but over time, to indicate population trends, target pest species, and also associated pest species.
3. Information that relates a level of use of a pest control method to the known side effects in order to ascertain changes in side effects resulting from different levels of pest control methods. From this kind of information estimates of the damage prevented as a result of the changes in control could be developed for products by regional products by pest. In addition, estimates of pest population changes due to the control change could be generated and, finally, changes in side effects as a function of changes in control could be estimated and related to yield damages prevented.

FACTORS INFLUENCING CHANGING USE PATTERNS

New pest problems that can have an effect on pesticide usage come about through several means. Many pest problems develop from new introductions either coming into the United States or from one area to another within the country. The introduction of such insect pests as the gypsy moth, and more recently the cereal leaf bettle, have had a significant effect on the use patterns of insecticides. When new pest problems are recognized, efforts are often initiated to restrict their migration usually through quarantines, and attempts are then made to eradicate the pest. Widespread control measures may become necessary when the new species become widely distributed.

Pests can develop a change in the preferred host plant and thus alter their economic significance. New problems which affect pesticide usage are created when previously undeveloped areas are utilized for public recreation. Such pest problems as disease-bearing mosquitoes and ticks and noxious weeds such as poison ivy and ragweed are typical of such situations.

When a new agricultural crop is produced more intensively new pest problems will often occur. Other occasions arise where an insect becomes a pest because factors such as temperature, moisture, food supplies, etc., are optimum to bring about a high population density. Examples of this are the various species of grasshoppers which in certain years build up to tremendous numbers and in other years may not be a problem.

Pest resistance.—The term "resistance" is applied to formerly susceptible species whose populations can no longer be controlled by a given pesticide at the rates normally recommended. The earliest instance of resistance in the United States was noted in 1908 when the San Jose scale resisted lime sulfur sprays in a few orchards in Washington. Early resistance to DDT was seen in the housefly by 1946 in Sweden. Fortunately, acquired resistance to DDT apparently does not involve a cross-resistance to cyclodiene derivatives, nor to organic phosphate compounds, and vice versa.

Resistance is a character developed by selection within a population of a species normally susceptible to a particular pesticide. It is an inheritable characteristic, developing only in populations that already have the factors for resistance, and not inducible by habitation during the lifetime of the pest organism.

Some 224 species of insects and acarines in various parts of the world have developed resistance to one or more groups of insecticides; of these, 127 are agricultural pests and 97 are pests of medical and veterinary importance. Resistance can be discussed in three major categories; DDT, cyclodiene, and organophosphate resistance. Of these three, DDT resistance occurs in 89 species, cyclodiene resistance in 116 species, and organophosphate resistance in 39 species. There are many populations in which two or three of the resistance factors are present simultaneously.

Since resistance is the result of Darwinian selection, it should be expected to develop wherever insects are exposed for long periods to selecting levels of the insecticide that causes some degree of mortality short of 100 percent. The change toward resistance will be more abrupt when the selecting level, in terms of percent mortality, is higher, and there will be less delay in its development when the area treated is wider and the surrounding untreated population is smaller. Residual insecticides are ideal selecting agents because they persist such a long period at selecting levels of contamination. The practical outcome of resistance to chlorinated hydrocarbon compounds has been the introduction of a variety of new organophosphate and carbamate compounds.

New pesticides.—To meet the residue problems associated with many uses of persistent pesticides, a wide range of organophosphate and carbamate insecticides has been developed. For example, malathion, carbaryl, and related materials are now used for the control of many insects where it is essential that crops or livestock are not treated with persistent materials. Ciodrin and dichlorvos are now available for the control of flies in dairy barns and milking areas, and for direct use on livestock. For gypsy moth control, DDT is being replaced in many areas by Sevin (carbaryl). Abate, a compound with selectivity for mosquito larvae, is replacing DDT in many areas.

New soil insecticides have replaced aldrin and dieldrin for certain agricultural insect control programs. Many newer organic phosphate compounds, such as phorate and disulfoton are applied as plant systemic insecticides in the form of granules.

New methods of application.—New application methods are being used to decrease the pesticide contamination hazards and to increase pesticide efficacy. Improved forecasting of outbreaks of pest infestations has often resulted in a reduction in the number of pesticide applications necessary for adequate control.

Direct incorporation of pesticides into soil has resulted in a reduction of such hazards as spray drift and residue on standing foliage. By direct furrow soil application, a smaller amount of pesticide can be used and still provide effective control.

The current interests in the use of chemical attractants and chemo sterilants involve using baits which, in some instances, can control the insect pest without contaminating the environment. The chemosterilants and the poisons used with the attractants are moderately toxic to mammals but there are newer ones on the way that are expected to be much safer.

Alternate control techniques.—Mechanical and cultural control measures are associated with normal agricultural procedures and generally involve certain changes in normal farming techniques rather than the addition of special procedures. Although widely used in the past, many cultural control techniques have been replaced by labor-saving chemicals.

Rotation of crops is often an efficient way to reduce weeds and insects. Another cultural practice that has been very effective is the development of insect and/or disease-resistant plant varieties. In general, breeding for pest resistant varieties is extremely slow and tedious and must be directed at a single type of pest. To be effective, resistance to a given disease or insect must be combined with desirable agronomic or horticultural characteristics.

The successful use of biological control techniques has been responsible for the control of a limited number of pest species. Most successful cases to date have involved the use of parasitic or predaceous insects. There are at least 18 such successful examples in the United States where noxious insects have been controlled by other insects.

Microbial control of certain insect pests has received increased attention in recent years. Nematodes, protozoans, bacteria, fungi, and viruses have been tested experimentally.

Availability of materials to work with is the limiting factor in using pathogens in control programs. There are also problems of registration of labels and quality control assays which need to be worked out for each organism.

The potential use of chemosterilants on a wide scale to control insect populations is an intriguing one. The development of suitable compounds, acceptable application methods, and a better understanding of insect habits are areas of research which must be fully explored before any serious effort can be made to use chemosterilants on a widespread commercial basis. It is likely that through continued research by universities, government agencies, and the chemical industry, relatively safe and specific chemosterilants will be developed for field use.

The potential uses of pheromones and other insect attractants fall into two categories: (1) population density surveys and (2) direct behavioral control. Although early work has been promising under controlled conditions, there are many questions yet to be answered before pheromones will have a major role in insect control.

Availability of Labor.—The availability of labor in agriculture today is forcing a tremendous push toward completely mechanized farming. It is a well-known fact that the trend is toward bigger and fewer farms, and it is becoming more and more uneconomical to employ hand labor to care for and harvest crops. As a result, there is a greater reliance on pesticides.

Economic Pressures.—Economic pressures are put on the grower from a number of sources.

The public demands top quality produce and the grower must meet these demands to obtain the premium market price. This requires protection of the produce from planting through harvest and until it reaches the consumer.

Certain regulatory pressures concerning contamination of processed foods with pest fragments have a strong influence on pesticide usage in order to reduce or eliminate the pests.

PESTICIDE GROUPS AND GENERAL USES

INSECTICIDES AND MITICIDES

Insecticides may be classified in several different ways. One system that has been widely used is based on the mode of entry of the insecticidal agent into the insect—stomach, contact, and fumigant poisons. Stomach poisons are materials which are ingested by the insect and kill primarily by action on or absorption from the digestive system. Their effectiveness is generally limited to the control of chewing insects. Contact poisons are absorbed through the body wall and must come into direct contact with the insect to kill. They are usually required against sucking insects. Fumigants enter the tracheal or respiratory system in the form of a gas and are effective against insects found within an enclosure.

Because of the large degree of overlap found when using this system of classification, insecticides are more frequently discussed in terms of their chemical nature. The major divisions are inorganic and organic. Organic insecticides are further broken down into oils, botanicals, and synthetic compounds. The synthetic compounds are by far the most widely used and are further subdivided on the basis of their chemistry.

1. *Inorganic Insecticides.*—Although inorganic insecticides have largely been replaced by more efficient organic compounds, some still find a place in agricultural pest control. Lead arsenate is used primarily on trees and shrubs to control chewing insects. It may also be used in baits for the control of ants and cockroaches. Other inorganic insecticides occasionally used are: calcium arsenate, various sulfur derivatives, and Paris green. Their general use is restricted because of toxicity to man, persistence, and the advent of newer and better insecticides.

2. Oils.—These are used in an emulsion and are employed as insecticides in a number of ways. They may be used as solvents or carriers for insecticides, such as diesel fuel in aerial applications. Oils also serve to carry insecticides over water for mosquito control or even oil alone may be used for this purpose. Highly refined oils, which are relatively nonphytotoxic, are applied to tree foliage. These are known as summer oils and are effective in controlling aphids, mites, and scale insects on fruit trees. The dormant oils, which are less refined, are restricted in use to application when no foliage is present. They are effective in eliminating over-wintering eggs of mites and aphids, and in controlling scale insects.

3. Botanicals.—A number of plant extracts are in active demand as insecticides despite the variety of synthetic organic compounds now available. These extracts, or botanicals, break down into harmless compounds soon after application and with a few exceptions may be handled with relative safety. They are quite specific in their effectiveness, being limited largely to soft-bodied insects such as aphids, thrips, and certain caterpillars, particularly the younger stages. The more important toxicants include pyrethrins, rotenone, and a few related compounds. All are of complex structure, and there has been little success in their development by synthesis, with the notable exception of allethrin, which is a synthetic "pyrethrin."

Pyrethrin and allethrin are formulated as dusts, sprays, and aerosols, usually with a synergist to increase insect toxicity. They are noted for rapid knockdown of insects through action as a nerve poison. Their low mammalian toxicity makes them very suitable for use around livestock and as household sprays. Rotenone is a selective insecticide that kills by inhibiting oxygen utilization by the insect. It may be used with relative safety around most animals, although swine are highly susceptible to its toxic action. Its greatest use as an insecticide has been for control of cattle grubs and external pests of livestock. Other botanicals of lesser importance include sabadilla, ryania, barthrin, dimethrin, and nicotine.

4. Synthetic Organic Insecticides and Miticides.—The synthetic organics dominate the insecticide field today. Rapid developments makes an up-to-date classification difficult, but they can be broadly grouped into general chemical classes.

a. The organochlorines or chlorinated hydrocarbons have been widely used since 1945. The outstanding feature of this group is the prolonged residual effect by both contact and stomach action. They are essentially insoluable in water, and have little or no tendency to be absorbed systemically into the plant. They have shown effective persistence for over 10 years in tests where massive soil treatments were used as in termite control. Contrary to popular belief, the organochlorines are rather specific in their action, being highly poisonous to insects in certain groups, and comparatively ineffective in killing others.

Resistance to these insecticides has developed in a growing number of pests during their period of use. The development of resistance to one organochlorine is usually followed by resistance to others. Hazards to applicators are minimal when these insecticides are used according to directions with the exception of endrin which must be handled with extreme care.

The problem of illegal residues persisting after harvest usually comes from this group of insecticides.

The most widely used organochlorine, as well as the most publicized, is DDT, which belongs to a class of compounds known as *diphenyl aliphatic chlorinated hydrocarbons.* Other compounds related to DDT are: Rothane (TDE), Marlate (methoxychlor), Kelthane (dicofol), Acaraben (chlorobenzilate), Acaralate (chloropropylate), as well as a few more of lesser importance, including Dimite, Karathane (dinocap), Bandane, and Dizane. These compounds are used at rates of 1 to 2 lb/A as insecticides and 2 to 4 lb/A as miticides.

A second class consists of *chlorinated aryl hydrocarbons.* This group contains a number of widely used compounds again mostly in agriculture. Examples are benzene hexachloride, chlordane, heptachlor, aldrin, dieldrin, endrin, endosulfan, toxaphene, and several more of lesser importance. Of these compounds, chlordane, aldrin, and dieldrin are widely used in structural pest control; e.g., termites.

b. *Organophosphorous insecticides.*—Organic phosphates have a wide range of insecticidal effectiveness. They are mainly contact insecticides, although many have fumigant action.

The organic phosphates act as inhibitors of the enzyme, cholinesterase. Often the effect is not immediately obvious and a worker may be exposed to the poison on successive days without apparent ill effects. However, grave symptoms appear when the critical level of enzyme inhibition is reached. The hazards of using the phosphates vary widely, depending on the compound, but they are generally considered to be more toxic than many of the organochlorines.

The phosphates as a whole do not have a long residual action. This makes some undesirable where a long period of protection is needed, but many of the phosphates are most important where residue tolerances limit the choice of available insecticides, and in control of insects resistant to chlorinated hydrocarbons.

Agriculture provides the major market area for the organic phosphates although a few are of importance in the area of public health (dichlorvos, Abate, and fenthion). A few also find limited use in home and garden products. *Heterocyclic derivatives* of phosphorous compounds are applied in the range of 1 to 5 lb/A. Examples are: Co-Ral (coumaphos), Dursban, diazinon, Guthion (azinphosmethyl), and several others of lesser importance.

A second group of organic phosphates is the *phenyl derivatives*. Examples of this group are: Nitrox (methyl parathion), Thiophos (parathion), Ronnel, Baytex (fenthion), Abate, Ciodrin, plus a large number of lesser importance. Application rates of these compounds are typically in the range of 0.5 to 2 lb./A.

The third class of organophosphorous compounds is the *aliphatic derivatives*. This group includes such compounds as Dylox (trichlorfon), Dibrom (naled), Vapona (dichlorvos), Phosdrin (mevinphos), Bidrin (dicrotophos), Systox (demeton), Thimet (phorate), Meta-Systox-R (oxydemetomethyl), malathion, Cygon (dimethoate), plus a number of others. These compounds are recommended to be used in the general range is 1 to 5 lb./A.

c. *Carbamate insecticides.*—The *carbamate* insecticides are of comparatively recent development and represent a unique class of insecticidal compounds of considerable diversity. These apparently owe their activity to action against the enzyme, cholinesterase, as do the organophosphates. However, unlike the phosphates, they are competitive rather than irreversible inhibitors of this enzyme. They are rapidly detoxified and eliminated from animal tissues and, thus are not accumulated in fats or excreted in milk. One of the surprising features is the synergistic action of carbamates which results from their combination with piperonyl butoxide, sesamex, sulfoxide, MGK 264, and other materials used as pyrethrin synergists.

The carbamates act by contact or stomach poisoning and are not fumigants or vapor toxicants. Their major area of use is on agricultural crops where recommended application rates range from 1 to 10 lb./A. The chief exception is Baygon, which is restricted to use by pest control operations for spot treatment in the control of cockroaches and other pests of public health importance. Other examples of carbamate insecticides are: Bux-10, Furadan (carbofuran), Lannate (methomyl), Sevin (carbaryl), Zectram, and several others of lesser importance.

d. Miscellaneous insecticide compounds.—There are several insecticides available that do not fit in the above-mentioned groups. They typically have a more limited activity spectrum and tend not to be as widely used. This group includes such materials as creosote and pentachlorophenol, which are used primarily to prevent termite damage to fence posts and foundations. Compounds of the nitrophenol group, such as dinitrocresol and dinitrobutylphenol have limited use in agriculture at 1 to 3 lb./A.

Several fumigants are available and are used both in agriculture and for public health. These include methyl bromide, hydrogen cyanide, and para-dichlorobenzene, which are applied at about 1 lb./1000 cubic feet of area. Fly sprays for use around the home and also in livestock structures may contain organic thiocyanates, Lethane, or Thanite.

Fungicides and bactericides.—These chemicals are toxic to fungi and bacteria. With plant diseases, these chemicals act to prevent the plant from suffering detrimental effects of the particular disease. To be effective, the fungicide or bactericide must be capable of preventing a disease from becoming established, or arresting the disease if it is already present. To accomplish this, an effective material to be used on crops or desired plants must have four attributes, in addition to relative safety to the crop and low hazard to the consumer of the product and applicator of the compound: (1) The material must be able to penetrate the microbial membrane or change these membranes to establish itself at the active locus; (2) it must enter into reaction with normal cell metabolism to disrupt the biochemical processes of the cell essential to its growth and functioning; (3) the toxicant must be selective so it will not enter into extraneous reactions in the plant cell and become detoxified or become attached to relatively inert cell structures such as spore wall; (4) the molecule must be sufficiently stable to permit its effective use as a spray deposit, chemotherapeutant, or as occurs in a few examples, to generate fungitoxic decomposition products as required.

The discussion of fungicides and bactericides in use today can best be accomplished through a classification based on chemistry. As with other pesticides, antimicrobial action can be related to molecular composition.

1. The *inorganic fungicides* are the oldest known fungicides and, despite the onset of new synthetic organic compounds, are still relied on by many growers. Their use is almost completely restricted to agriculture, with some application in the golf course and turfgrass industry, but with only minor penetration in the home and garden market. Recommended rates range from 1 to 20 lb./A. Examples of inorganic fungicides on the market are: Bordeaux mixture, copper sulfate, copper oxide, copper zinc chromate, sulfur, lime sulfur, mercuric chloride, Clorox, cadmium chloride, plus a few other related compound mixtures.

2. Considerable effort has been expended on the development of *antibiotics* as fungicides and bactericides to control diseases of plants. A number of effective materials have been discovered but have not been reduced to commercial use. One of the major problems has been phytotoxicity. Agrimycin (streptomycin) is a bactericide that has proven itself to be an effective control agent when used anywhere from 0.5 to 200 lb./A depending upon the target bacterial disease. Actidone (cycloheximide) exhibits similar activity as a fungicide when used in a range of 5 to 100 grams per 100 gallon spray solution.

3. *Organic mercury compounds* are used as fungicides for seed treatments and for bulb and corm treatments. A few organic mercury compounds are used as foliar sprays. Areas of use are chiefly agriculture with a small market in the specialty area; i.e., golf courses and sod industry. Examples of products using organic mercury formulations are: Phenyl mercuric acetate, Semesan, Ceresan M, Panogen, Chipcote 25, Emmi, and Memmi. Recommended treatment rates of these compounds range from 0.25 to 5 ounces per bushel of seed or 0.5 to 5 lb./A.

Several *metal organic fungicides* are available and used chiefly for treatment of handling, harvesting, and storage equipment. They also are used to prevent rot and mildew in wood and fabrics. These products are recommended to be applied to a 1- to 5-percent solution. Examples are: Copper naphthenate, copper oxinate, quinolinolate, and Du-Ter.

5. The *dithiocarbamate fungicides* have their greatest use for foliar disease control of agricultural crops. Many are also effective when used as a soil drench. Recommended rates of application range from 1 to 15 lb./A. They are, for the most part, the metallic salts of dithiocarbamic acid derivatives. The metallic salt form provides them with the necessary stability to remain effective long enough to control the

target disease organism. Examples of dithiocarbamates being used today are: Vapam (SMDS), Zerlate (ziram), Parzate (nabam), Manzate (maneb), Dithane Z-78 (zineb), Arasan (thiram), Fermate (ferbam), and Polyram (metiram).

6. Chlorine-containing fungicides are effective against a large number of agricultural crop diseases. They may be used as foliar sprays, soil drenches, seed treatments, or dormant sprays. They are effective against turf diseases, powdery mildew, scab fungi, and several other pathogens. Depending on the compound, they are used at rates ranging from 0.25 to 5 or 10 lb./A. Examples of this class of fungicides are: Penta (PCP), Terrachlor (PCNB), Hexachlorobenzene, Captan, Difoltan, Phaltan (folpet), Phygon (dichlone), Lanstan, Spergon (chloranil), Dyrene, Daconil 2787, Terrazole, Demosan, and Botran (dichloran).

7. There are a number of fungicides of variable chemical nature that fall into a miscellaneous grouping. They are used both in agriculture and in the sod and turf industry. Recommended rate ranges are from 0.2 to 3 lb./A. Examples are: Karathane (dinocap), Morocide (binapacryl), Dexon, Cyprex (dodine), Diphenyl, Dowcide A, Glyodin, Morestan, and Creosote.

Herbicides, defoliants, and desiccants

There are almost 100 different chemicals and combinations of chemicals that are used effectively as herbicides. There are three basic types of herbicides depending upon their effects on plants: Contact, systemic, and soil sterilants.

Contact herbicides kill plant parts through direct contact with the foliage. Generally the effects are acute and the plant dies quickly. Contact herbicides may be selective in their action or they may be nonselective and kill all plants.

Systemic herbicides can be absorbed by either the foliage or the roots and may be translocated through the entire plant system. They are usually selective in their toxicity and they usually have a chronic effect on susceptible plants.

Soil sterilants are chemicals which prevent plant growth when present in the soil. The length of time for effectiveness may range from less than 48 hours to more than 2 years.

The greatest area of use of herbicides is in agriculture, although, considerable amounts are used in maintenance of rights-of-way, waterways, maintenance of industrial areas, in the home lawn and garden, and by various Governmental agencies.

1. *Inorganic herbicides* are derivatives of inorganic acids where hydrogen has been replaced by a metal. In sufficient concentration these provide a contact burning effect. The rates of application are usually

high, ranging from 100 to 1,200 lb./A. Examples are: Sodium arsenite, calcium arsenate, sodium chlorate, and sodium borate.

2. *Metal-organic compounds* include those having a metal ion complex combined with an organic portion of the molecule. These compounds are usually used to control large areas of weeds such as on railroad and highway rights-of-way. A few are selective and are used to control crabgrass in desirable turf lawns. Examples are: Disodium methane arsonate, cacodylic acid, phenyl mercuric acetate, and several other similar products.

3. *Carboxylic aromatic herbicides.*—This large group of synthetic herbicides has chemistry with two characteristic moieties, a *carboxyl* group and an *aromatic* group. Their activity includes contact, systemic, and soil sterilant action, depending upon the compound and the rate and method of application. They can be divided into five types: Phenoxy acids, phenylacetic acids, benzoic acids, phthalic acids, and phthalamic acid herbicides.

a. *Phenoxy herbicides* are a selective group of compounds used for broadleaf weed and woody plant control. They are systemic in nature and in warm moist soil persist 30–60 days. They are only slightly toxic to man and other animals. Examples are: 2,4-D, 2,4,5-T, silvex, sesone, MCPA, erbon, dichloroprop, and others. The recommended rate range for use is from 0.25 to 2 lb./A.

b. *Phenylacetic acid.*—The single *phenylacetic* acid of note is fenac. It is used at 4 to 20 lb./A for a variety of purposes including agriculture, aquatic weed control, and right-of-way weed removal. It is more persistent in the soil than the phenoxy herbicides but would not be expected to accumulate from 1 year to the next. It also has a low toxicity to mammals.

c. *Benzoic acid compounds.*—The *benzoic acid herbicides* have a longer soil persistence than the phenoxy compounds and have a low toxicity to mammals. Their major use is in agriculture where they are effective against annual and perennial broadleaves and grasses. Several show some crop selectivity. Examples are: Amiben, Banvel D (dicamba), Benzac (PBA), and Trysben (2,3,6-TBA). Application rates range from 1 to 4 lb./A.

d. *Phthalic acid compounds.*—The *phthalic acid herbicides* are preemergence herbicides that prevent weed germination. They are persistent for only about 30 days in the soil and they are relatively nontoxic to mammals. They are used in agriculture at 6 to 12 lb./A. Examples are: Dacthal (DCPA) and endothall.

e. *Phthalamic acid compounds.*—The *phthalamic acid herbicides* are preemergence compounds with selective activity. They are applied at 2 to 8 lb./A and are used almost exclusively for agriculture. They are relatively safe to humans and other warm-blooded animals. No residual toxicity is expected to remain in the soil from one year to the next. The best example is Alanap (naptalam or NPA) which is available in several forms.

4. Aliphatic Acid Herbicides.—The chemicals in this group are *aliphatic compounds* containing a carboxyl group. They are grass killers with limited toxicity to broadleaf species. At low rates of 3–6 lb./A they are agricultural herbicides, but at 10–50 lb./A they are temporary soil sterilants. They are only slightly toxic to humans and warm-blooded animals and present no health hazard under normal use. Examples are: Dowpon (dalapon) and trichloracetic acid (TCA).

5. Substituted Phenol Herbicides.—*Substituted phenols* are used for contact killing of all weeds hit by the spray. They are applied to railroad and highway rights-of-way and industrial areas as well as on agricultural crops. They are also used as preemergence herbicides. Their persistence in the soil is only about 3 to 5 weeks, and they are not translocated in the plant. Examples are: dinoseb (or DNBP) and pentachlorophenol. Their toxicity to mammals is considered moderate to very toxic.

6. Heterocyclic Nitrogen Derivative Herbicides.—The *heterocyclic nitrogen derivatives* are agricultural herbicides with low mammalian toxicity. When applied at 1 to 4 lb./A, they demonstrate good selectivity, some possessing preemergence and some postemergence activity. At higher rates of 10 to 40 lb./A, a few are effective soil sterilants. At rates of less than 4 lb./A and under a warm, moist environment they seldom persist in the soil for more than 1 year. Examples are Aatrex (atrazine), Princep (simazine), Milogard (propazine), prometone, and amitrol.

7. Aliphatic Organic Nitrogen Herbicides.—*Aliphatic organic nitrogen* compounds can be subdivided into three general types: the substituted ureas, carbamates, and other amides.

a. Substituted Ureas.—*Urea* is a common agricultural nitrogen fertilizer. Replacement of some of the hydrogen atoms in the urea molecule with other substituents has provided a number of effective herbicides. They are absorbed easiest through the roots and will normally persist in the soil 3 to 6 months at preemergence rates (1 to 4 lb./A) and up to 24 months at soil sterilant rates (20 to 50 lb./A). They are relatively safe to warm-blooded animals and fish when used at agricultural rates. Examples are: Telvar (monuron), Karmex (diuron), Dybar (fenuron), Lorox (linuron), Cotoran (fluometuron), Tenoran (chloroxuron), Herban (norea), and Tupersan (siduron).

b. Carbamates.—A number of *carbamates* have been proven to be quite effective as agricultural herbicides when used at rates ranging from 1 to 6 lb./A. They are most effective in preemergence applications, and are relatively nonpersistent in moist, warm soils. Although a few carbamate herbicides will cause dermal irritations, they are considered to be moderately safe to humans and warm-blooded animals. Examples are: Vegedex (CDEC), Chloro IPC (CIPC), Eptam (EPTC), Tillam (pebulate), Sutan (butylate), Vernam (vernolate), Carbyne (barban), Ordram (molinate), Avadex (dillate).

c. Other Amides.—There are several amide derivatives used as herbicides in agricultural and home and garden products. They are basically preemergent herbicides with a soil persistence of 1 to 3 months at the recommended rates of 1 to 10 lb./A. For the most part they are only slightly toxic, but one, CDAA, is dermally toxic. Other examples are: Dymid (diphenamid), Betasan (bensulide), Lasso, dicryl, Clobber (cypromid, and Stam (propanil).

8. The *dinitroaniline herbicides* are preemergence compounds effective against annual grasses and some broadleaf weeds. They are extensively used in field and horticultural crop weed control and turf. At the recommended rates of 0.5 to 3 lb./A, in warm, moist soil they have a persistence of 2 to 6 months. Their toxicity to humans and warm-blooded animals is only slight, making them safe to handle. Examples are: Treflan (trifluralin), Balan (benefin) and Planavin (nitralin).

9. The *nitrile herbicides* are used for preemergence broadleaf weed control in small grains and also in orchards and nurseries. Moderately toxic to mammals, they range in soil persistence from 1 month to over 2 years. The recommended application rates vary from 0.5 to 15 lb./A, depending upon the compound and desired use. Examples are: Buctril (bromoxynil), Certrol (ioxynil), and Casoron (dichlobenil).

10. Herbicides falling into a miscellaneous category include uracil derivatives, chlorinated compounds, aldehydes, and others. The group includes at least two compounds, paraquat and diquat possessing higher mammalian toxicity. Others of this group are considered moderately toxic. They are used in agriculture as well as in industry. Other examples include: Tordon (picloram), propachlor, Aqualin (acrolein). Pyramin (pyrazon), Sinbar (terbacil), and bromacil.

Nematicides

Nematode control requires the use of clean soil, clean planting stock, and sanitation. Chemicals used to kill nematodes must not only be efficient for killing the organism, but also must leave no residues harmful to plants. Preferably they should be easy to apply. The most effective nematicides have been those with fumigant action. The fumigant action may come from a gas confined at the soil surface or from volatile liquid or granular compounds actually placed in the soil. All are poisonous to man and animals.

1. Halogenated Hydrocarbon Nematicides.—These nematicides may be injected into the soil or they may be gaseous fumigants applied to the soil surface under gasproof covers. They have a residual activity of about 1–6 weeks during which time they can be harmful to plants. They should be regarded as moderately to very toxic to the applicator. Many are mixtures of two or more active compounds. Examples are: Dowfume MC–33 (methyl dibromide+chloropicrin, D–D (dichloropropane+dichloropropene), Telone (mixture of chlorinated C_3 hydrocarbons), Nemagon (dibromochloropane), and ethylene dibromide. Rates of application recommended for these compounds are 1 to 2 lb./100 ft.^{2} of fumigant or 12 to 20 gal./A of soil injected liquid.

2. Organic Phosphate Nematicides.—A special formulation containing 4 lb./gal. diazinon controls soil insects and ecto-parasitic nematodes of southern turf grasses. This product, Sarolex, has a recommended application rate of 10 to 20 lb./A.

3. Cyanate Nematicides.—Vorlex is a preplant soil fumigant that controls weeds, fungi, insects, and nematodes. It is a mixture of methyl isothiocyanate and chlorinated C_3 hydrocarbons. This mixture is applied at about 20 gal./A. At least 2 weeks must be allowed before planting a crop. No cover or water seal is required.

4. Thiophene Nematicides.—An example of a thiophene nematicide is Penphene (tetrachlorothiophene, or TCTP). It is recommended for controlling a number of nematode pests on tobacco. It kills by fumigant action when injected into the soil at 6 to 8 lb./A. After 2 weeks the soil must be aerated before the crop is planted.

Rodenticides and mammalian biocides

The interrelationships of man and animals have become increasingly complex as human populations have increased. As wildlife habitats have become altered, certain species have established new balances. Thus some species have substantially increased in population density, often creating problems that adversely affect man's interests and welfare. There are numerous situations when control of predators and rodents is essential to protect agricultural and pastoral interests as well as human health and safety.

Of the various kinds of nuisance, destructive, disease-carrying, or predatory mammalian pests, rodents are the main targets of control. A number of chemical control measures have been developed for rodents, many of which can be used on other mammalian pests such as coyotes, skunks, raccoons, etc. Compounds which are toxic to rodents are usually also toxic to humans and should be handled with utmost caution.

1. Inorganic rodenticides.—There are a number of inorganic compounds that are effective against such pests as rats, mice, moles, and gophers where they are problems. Although most often used as 1 to 2 percent baits, a few are active as fumigants. Examples of some inorganic rodenticides are: arsenic trioxide, arsenic sulfide, barium carbonate, calcium cyanide, zinc phosphide, thallium sulfate, and sodium fluorosilicate + zinc cyanide.

2. Botanical rodenticides.—Certain plant extracts have toxic activity toward rodents when used in baits at 0.5 to 1 percent. One of these, red squill, is specific for rats and nontoxic to other warm-blooded animals when used at specified rate ranges. Strychnine in both the alkaloid and sulfate form is used chiefly in poison baits set for squirrels, gophers, rabbits, and some lesser pests.

3. Anticoagulant rodenticides.—The anticoagulant rodenticides are highly effective in controlling rats and house mice. They are essentially odorless and tasteless and are effective in low doses. Action is not rapid and usually 3 to 5 feedings from a 0.5 percent bait is required. Death is due to internal hemorrhaging. These baits are recommended for use only in protected situations where access by higher animals is prevented. Examples of this class are: Fumarin (coumafuryl), Diphacin (diphacinone), Warfarin (coumafene), Pival (2-pivalyl-1,3-inandione), and Valone (2-isovaloyl-1,3-indandione).

4. Fluoride Rodenticides.—The fluoride rodenticides are extremely toxic to warm-blooded animals and their application is restricted to use by licensed pest control operators. They are odorless, tasteless, and fast acting, chiefly affecting the heart, with secondary effects on the central nervous system. Two such compounds are in use: Compound 1080 (sodium fluoroacetate) and Fluoroakil 100 (fluoroacetamide).

5. Miscellaneous Rodenticides.—There are a few other rodenticides available with a greater degree of selectivity toward rats. One of these, ANTU (2-napthylthiourea) is specific for the Norway rat when used as a 1 to 2 percent bait. Another is a specific single dose rat poison for Norway and roof rats. This compound, Raticate (norformide), is said to be nontoxic to a large number of other warm-blooded animals.

Molluscicides

There are several invertebrate poisons used to control molluscs where their presence is undesirable. They are relatively specific and are essentially nontoxic to fish and warm-blooded animals.

Polystream is a mixture of chlorinated benzene fractions that is effective in oyster beds to control oyster drill, a predatory snail.

Bayluscide (chlonitralid) is used to control snails and lamprey in flowing streams.

Matacil (aminocarb) is a carbamate insecticide that is also very effective in controlling garden snails and slugs.

Metaldehyde is a component of slug and snail baits that acts both as an attractant and toxicant. It may be formulated with or without calcium arsenate.

Piscicides

The use of piscicides is restricted to game and fish management for improvements to public waters. The main objective is to remove rough or trash fish prior to restocking lakes and rivers with more desirable game fish.

Most widely used for this purpose is rotenone, a highly toxic fish poison. Used in low dosages, it stuns the fish. As the fish surface, desirable species are seined out and placed in fresh water tanks where most revive. A second lake application kills undesirable fish.

The antibiotic antimycin is finding increasing use as a fish toxicant.

Lampricide L–30–F (3-trifluoromethyl-4-nitrophenol) has been used extensively in stream management to control populations of lamprey.

Toxaphene has also been used on occasions as a means of killing undesirable fish in water management programs though it is not registered for the use and the manufacturer discourages it.

Avicides

Some of the most difficult control problems concern birds in cities, at airports, around homes, and those which gather in great flocks and cause damage to grain crops, animal feedlots, truck and fruit crops. Some of the more troublesome species are pigeons, gulls, starlings, and blackbirds.

Avitrol (4-aminopyridine) controls nuisance and destructive birds as a treated grain bait. It causes individual members of the flock to utter distress cries which, in turn, causes other members of the flock to leave and avoid the "undesirable place." It is used to control pigeons around city buildings, gulls at airports, and starlings in feedlots.

Another compound, Queletox (a formulation of Baytex insecticide), is also used to repel birds by affecting a few individuals which frighten others away.

Also used for bird control because of their repellent action are anthraquinone and thiram. Thallium sulfate may be used in a bait to control starlings but is restricted to use by Government agencies because of dangerous cumulative poisonous properties.

Related Materials and Their Uses

REGULATORS OF PLANT GROWTH AND REPRODUCTION

The control of plant growth and differentiation through the use of chemical substances is a new development. A number of chemicals are now known that have a relatively broad spectrum of effects while others merely mediate or block specific metabolic pathways. As a general class of compounds, the plant growth regulators are considered to be relatively nontoxic to humans and other warm-blooded animals. Their persistence in soils is short, only 4 to 6 weeks.

Auxins.—Synthetic auxins were the first chemical regulators to find widespread agricultural use. Rate levels of 10 to 50 ppm are useful for promoting rooting of cuttings, setting of fruit, fruit thinning, delaying preharvest drop of fruit, and for control of flowering of pineapple. Examples of synthetic auxins in use today are: β-indolebutyric acid, m-napthaleneacetic acid, β-napthoxyacetic acid, *p*-chlorophenoxyacetic acid, 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-T, and silvex.

Gibberellins.—A large number of varied responses in plants are associated with the gibberellins. There is marked stem elongation; overcoming of physiological and genetic dwarfism; acceleration of flowering in cold-requiring biennials and long-day plants; promotion of fruit setting; fruit enlargement; elimination of seed, bud, and vegetative dormancy; promotion of lateral bud growth; seed germination; and growth at suboptimal temperatures. Rates of application vary with the effect desired and may be from 1 to 1,500 ppm. The chief example is gibberellic acid (gibberellin A_3).

Cytokinins.—These compounds are of relatively recent origin with their use potential being limited to favorable effects of prolonging storage life of green, leafy vegetables and possibly in the enhancement of fruit setting. They are applied at rates of 5 to 10 ppm and are often combined with one of the other kinds of growth regulators. Examples of the cytokinin group are: N^6-benzyladenine, N^6-benzyl-9-tetrahydropyranadenine, and zeatin.

Growth Retardants. The growth retardants, or inhibitors, may become useful in the growing and handling of crops and in reducing woody plant growth. They inhibit sprouting of onions and potatoes in storage; they increase wheat yields by increasing tillering and stimulating shorter and thicker stems to reduce lodging. A few are used extensively to control growth of flowers and ornamentals. They may increase cold resistance. Examples of growth retardants are: maleic hydrazide, Cycocel, Alar, and Phosphon.

Chemical Repellents

Repellents have two principal advantages: since they need not kill the pest species, the best repellents have low general toxicity and may be used safely on man, beneficial animals, and food plants; and they can provide protection for an individual man, animal, or plant without the necessity of destroying a huge segment of the pest populations, with all the expense, difficulty and even hazard that this may involve. Repellents also have some disadvantages. Because the pest population is not destroyed, but only held at bay, the host must be completely and continuously covered with the repellents to obtain protection. Usually the repellents that protect man and animals are lost rapidly by abrasion, evaporation, and absorption through the skin, necessitating re-treatment at intervals of a few hours or days at most.

The uses of repellents include protection for domestic animals from biting flies. These may be combined with a low level of pyrethrins. Man uses repellents to help ward off biting and disease-carrying insects such as mosquitoes, flies, and ticks. Repellents are also used to prevent pests from infesting certain areas such as food and drink containers, as well as termite-susceptible structures.

Some insect repellents available are: Ethyl hexanediol (6–12), N, N-diethyl-m-toluamide, butoxy propylene glycol, dibutyl succinate, and octyl propyl sulfoxide.

Attractants

Chemical attractants and associated agents serve useful purposes as lures in traps. As insect attractants they are used to detect pest infestations, estimate population densities and aid in control of the pest either through traps or incorporated into poison baits.

Some chemical attractants are biologically active in extremely small quantities. This is particularly true for those associated with sexual behavior. Others are apparently feeding attractants. Although not always true, insect attractants are usually quite specific for a given species and often for a single sex of the species.

There are at least seven synthetic attractants available for use in control of fruit flies and melon flies. They are methyleugenol, anisylacetone, cuelure, siglure, medlure, and trimedlure. These have been very effective in aiding with Mediterranean fruit fly control. Other attractants are gyplure, bombykol, butylsorbate, and methyllinolate.

LEGISLATION AND REGULATION RELATING TO PESTICIDE PRODUCTION AND USE

Experimental labels, as currently administered, offer an opportunity for a manufacturer to place a relatively small amount of a product on the market in order to better determine the acceptance of the product in the market and the need that it may or may not fulfill in the marketplace. The advantages of this approach are that:

1. The manufacturer can get an early assessment of the future of the product which may help him determine the amount of investment

he must make in new manufacturing plants and further product developments; and

2. It permits the regulatory agency to have a close look at any problems that may be encountered by the product. Efficacy or safety deficiencies will likely be brought out by the experimental label procedure without an overwhelming liability to the manufacturer or threat to the environment or populace.

In recent years experimental labels have been more widely used. Under present procedures it is necessary to account for all material placed under the experimental label and to provide biological results on the material used. This procedure is helpful, but a further step appears in order; namely, the limited sale in regular commercial channels without the requirement of biological results on the material should be permitted or even made mandatory for 1 year prior to a full registration. This would go even further toward enabling the manufacturer to assess the product in the marketplace but with a limited exposure on his part and would similarly provide the regulatory agency with a greater opportunity to assess possible threats to man or his environment. The experimental label is not now a mandatory procedure, though it is becoming much more widely used. If the limited label concept is instituted, it is proposed that the limited label be mandatory but that the experimental label remain discretionary on the part of the applicant.

Improved Label Clarity

Efforts should be made to achieve label clarity. The purpose of the label on pesticide containers should be to identify the product, explain the use, and provide adequate directions for use and sufficient protection for the applicator. Specific improvements recommended for greater clarity are:

1. The use of the product should be prominently displayed on the front panel in language familiar to the consumer; such as, "Weed Killer," "Insect Killer," "Growth Regulator," etc.

2. The ingredient statement should be simplified. It should use common names only (and every active ingredient should have a common name) with no reference to chemical names as a footnote outside the ingredient statement, no reference to licensing agreements, no patent declaration, and no conversion to equivalents within the ingredient statement. Otherwise, the style for the declaration of ingredients could remain identical to current requirements of USDA. The entire ingredient statement should be printed in such a way as to stand out from the rest of the label copy; i.e., printed with a contrasting background.

3. The label should specify what the product will and will not do. Organisms to which the product is hazardous should be indicated. For example, if a compound is known to be very toxic to specific types of animals such as fishes or birds this information should be highlighted on the label.

4. The label should be dated in some manner—date of manufacture; an expiration date based on product shelf life or stability; or, at the least, the date of label registration expiration.

5. It is recommended that an entirely new scheme of denoting relative toxicity be devised. The average consumer does not understand the progression from caution, warning, to poison. A graphic or numerical representation of the degrees of oral and dermal toxicity should be developed to enable consumers to select less hazardous materials. Professional graphic communicators should be consulted on this project. Cautionary statements should clearly indicate the hazards without undue cautionary statements.

6. The manufacturer should be responsible for providing, on the label, information on how to handle spillage and other accidents. Complete instructions on the disposal of excess material and empty containers should be provided.

Consideration of Experimental Stock in Commercial Channels

During the past quarter century of intensive organic chemical pesticide development, many products and product forms have been placed on the market. Today, with greater knowledge and better analytical tools, regulatory agencies would probably not allow some products on the market at all. Other products would require much more restrictive labels. The problem is especially acute in the area of homeowner products, where some highly toxic materials have been made available to the homeowner in small packages and where other products have borne unrealistically restrictive labels such as "Wear rubber gloves, mask and goggles," or "Wear protective clothing." Problems of shelf life, chemical stability, and physical stability have undoubtedly arisen in many of these products. Packaging materials of several years ago may be less satisfactory than would be permitted today.

At the present time, all pesticide products are registered with the USDA for a 5-year period. This expiration date does not now appear on the label. If it did, all obsolete stock not bearing a valid expiration date should be collected and disposed of. Collection, of course, causes the problems of financial responsibility to be brought forth. In many cases the product might be returnable to the manufacturer. In cases where companies have gone out of existence, some other means of indemnification at public expense should be considered. It is imperative that a realistic and workable collection and disposal system for both pesticide and containers be established, and it is suggested that our present county agent system for rural areas and public health officer system for urban areas might be the appropriate collection center. The removal of all obsolete stock from commercial channels would permit much better regulation of new products and would remove many potential hazards from the marketplace.

Problems of Introduction of New Pesticides—Costs

The development cost for any new pesticide is influenced by a number of factors. Some of these are related to the nature of the chemistry of the pesticide; but the most important influences on cost are those caused by the nature of our economy; i.e., labor costs, equipment cost, facilities overhead, etc., and the intended use of the new pesticide; i.e., food crop or nonfood-crop use.

Some insight into the cost of developing a new pesticide can be gained from a review in a recent publication on R & D costs which appeared in the April 26, 1969, issue of *Chemical Week*, page 38. The following table outlines costs for various stages in pesticide development and indicates the odds of reaching each stage.

Step	Average cost per compound	Chance of reaching next step	Cumulative	Total R. & D. cost
Synthesis and initial screening	$400	1:100	1:100	$40, 000
Toxicity testing	100, 000	1:10	1:1, 000	1, 000, 000
Field evaluation	400, 000	1:4	1:4, 000	1, 600, 000
Product development	200, 000	1:2	1:8, 000	400, 000
Process development and pilot plant	200, 000	1:1. 5	1:12, 000	300, 000
Test marketing	200, 000	1:1. 5	1:18, 000	300, 000
Commercialization	[1] 1, 000, 000	1:2	1:36, 000	2, 000, 000
Totals	2, 100, 400			5, 640, 000
Sales over $5 million/year		1:10	1:360, 000	

[1] This assumes no marketing organization has been established. Otherwise, the commercialization step would be reduced to $200,000. Source: Arthur D. Little. Figures are for 1964, latest year for which they are available.

The value given for "toxicity testing" appears to be low, particularly if a compound is to be used on a food crop. The overall figure of $2.1 million per compound appears to be a reasonable one. For pesticides to be used on nonfood crops, this figure can be somewhat reduced. The annual outlay for agricultural research by industry was reported at $473 million in 1965, which represented 55 percent of all funds spent by government and industry for this purpose (from the National Program of Research for Agriculture, USDA, 1966). Thus, the search for new, more effective pesticides is an expensive one.

Reduced incentives

An important part of every decision to develop a new pesticide is the competitive situation in the marketplace. Quite obviously a new compound which has better activity, is safer or more convenient to use, or is less costly than competitive pesticides, has economic advantages which may make the market attractive. However, in many cases these factors are unknown or difficult to determine accurately in the early stages of product development. Thus, a development decision is often made on the basis of limited knowledge of the true potential of the compound. Mistakes can be made which add to the development cost prorated against successful products.

When faced with decisions regarding the commitment of several hundred thousand dollars to compound development, research management often looks to the profitability of the market for the compound. When faced with such products as 2,4-D (selling wholesale at less than 40 cents per pound) and DDT (at less than 18 cents per pound), management has difficulty in justifying a large research expenditure for a compound that has similar biological activity. In addition, if adequate technical support is to be given a new product, there must be sufficient return to finance such support over at least the initial years of consumer use when he is learning to use the compound correctly. At the outset when the ability of the new product to compete with existing and perhaps lower cost materials is unknown, only those compounds possessing distinct advantages over existing products have a chance for success.

Increased regulatory requirements

Regulatory requirements can be expected to be increasingly more stringent as the years go by. As our technology advances, there will be many more questions to answer and requirements to be met. Each new pesticide not only must satisfy existing requirements but in itself creates new questions which must be answered.

In the quest to learn more about the impact of chemical pesticides on the environment, there will be new information developed which may alter present pesticide regulatory requirements. There will be more interest in persistence, metabolites in plants and soils, water and air pollution, etc. It can be expected that governmental regulatory agencies will constantly modify requirements with changing patterns of agricultural technology and cultural practices. Just as the science of agriculture changes, the regulations governing pesticide development and use will change as needed. We must insure that such changes are well founded and beneficial to agriculture and mankind. Every

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technological advance is associated with certain risks. In the final analysis the benefits to mankind must be weighed against the risks to mankind.

Multiple regulations

As Federal Government regulatory requirements increase, it is safe to predict that there will be greater interest on the part of State and local governments. California has been a leader in the development of a state regulatory program; other States have a good beginning, and still others will follow suit. In all probability most State regulations will follow Federal guidelines and will likely be less demanding.

Limited markets

Two types of limited markets can be envisioned, one governed by crop acreage, the other by competition. In the first situation, the crop acreage potential for the pesticide is so limited as to make the market too small for an economic return on research cost; e.g., a herbicide for garlic or turnip greens. In the other situation, a large number of efficacious, low-cost products on the market so limit the potential for a new material of equal or slightly better activity that a return on research investment cannot be realized.

Public reaction

While somewhat unpredictable, we can expect greater awareness by the public of pesticides and their use. Everyone in government and industry has a serious moral obligation to deal in facts to the public if pesticide and agricultural technology are to advance. Reporting and exaggerating the danger of pesticide usage without equal treatment of the beneficial aspects of pesticides threatens to retard the advancing technology required to meet food and public health demands around the world.

ADVANTAGES AND DISADVANTAGES OF SUBSTITUTE METHODS OF PEST CONTROL

There is growing concern about the distribution of pesticide residues in the environment, the effects of these residues on ecological systems, and possible effects on human health. Therefore, there is growing interest in alternative means of controlling pests. Some of the proposed alternatives themselves present hazards that should be carefully considered before they are put into widespread use. Our purpose here is briefly to consider some of the consequences of replacing present methods of control.

DDT is a very low cost material (17.5 cents per pound, 1968) and it is unlikely that any effective insecticide will be produced that does not cost more on a unit weight basis. However, any control method that does not have to be repeated frequently will cost less in the long run, and any that do not have the damaging side effects on the environment that DDT exhibits will have rather intangible values that can at least partially compensate for greater dollar costs.

It is felt that a reconsideration of cultural controls such as schemes of crop rotation and trends away from monoculture of single crops over huge areas is needed. It is widely taken for granted that such methods would add greatly to labor costs, but we are unaware of any cost-benefit analyses that have taken account of all relevant factors including the savings in pesticide costs and the intangible benefits.

More emphasis should be placed on the development of resistant varieties of crops. This is the only method of control that has proved feasible against the stem rusts of cereals, and the approach should be extended to other types of pests. Although, as with the rust fungi, pests are likely to evolve the ability to exploit resistant strains of crops, the availability of strains with different patterns of resistance to pests can greatly alleviate the need for other means of control.

Research should be encouraged on the possibilities of displacing pest species through competition with innocuous species. For example, most species of blackflies will not bite man. It is conceivable that some simple environmental modification could lead to displacement of noxious forms.

These approaches, together with quarantine regulations and the synthesis of insect pheromones which would be absolutely specific for a particular pest species, are not regarded as involving any risks of undesirable side effects. Some other proposed alternative controls require further discussion.

Alternative chemicals

As the chemical insecticides currently in use are removed because they have lost their effectiveness or have been recognized as hazardous, consideration will be given to replacing them with other chemicals that may be even more hazardous. For example, parathion is an extremely poisonous chemical that can be absorbed through the skin. It has caused a significant number of human deaths when used without proper precautions.

While most of the organophosphate pesticides usually break down quite rapidly in the environment they can affect various enzyme systems in man and domestic animals at very low concentrations. The effect of prolonged exposure to minimal residues of other pesticidal chemicals are unknown.

Very little is known about possible synergistic or antagonistic interactions of various chemicals. It is known that when dieldrin and

DDT are present simultaneously, each affects the storage of the other in fatty tissue. It has been reported that the presence of detergents in bodies of water prolongs the residual time of organophosphates. Pesticide residues are believed to have the potential for altering body responses to drugs. These interactions are essentially unpredictable and difficult to investigate experimentally, but are indicative of the inevitable risks involved in introducing new chemicals into the environment.

Biological control

The principal hazard of introducting predators into a new region is the possibility that the introduced species will itself become a pest. The mongoose was introduced into the West Indies for the purpose to control rats but it became a scrouge of poultry raisers and of the native ground nesting birds.

An outstanding success of biological control in Australia was control of the prickly pear cactus by a moth imported from Uruguay. In this case, prior to the introduction, Australian scientists carefully screened various potential control agents and rejected several because it was found that they would feed on garden crops if they ran out of cactus.

Care should also be taken with the introduction of bacterial and fungal diseases to be certain that they are not going to attack beneficial species. For example, the milky disease which is used against the Japanese beetle attacks beetles of only one family which includes no species known to be beneficial.

The great merit of biological control is that a one-time application may permanently solve a pest problem if sufficient care is taken in advance to be certain of the characteristics of the forms to be introduced. Another possibility which has not received nearly the attention it merits is the encouragement of native species which have the potential for aiding in pest control if ways can be found to encourage increases in their numbers.

The widespread use of broad spectrum chemical pesticides has sometimes had adverse effects on biological control. Scale insects which had been adequately controlled biologically have broken out as a result of predacious insects being killed.

The sterile male technique

The spectacular success of the program to eradicate the screw-worm fly in the Southeastern United States by releasing male flies that had been sterilized by radiation has focused a great deal of attention on this approach.

In the program against the screw worm fly the males were sterilized in the pupal stage in the laboratory by exposing them to gamma radiation. Now proposals are being made for sterilizing insects in the field with chemosterilants. Some of the chemicals proposed for use are powerful mutagens and carcinogens that may pose a threat to man and beneficial organism. Even if chemosterilization were performed in the laboratory, there seems to be no information about possible effects on predators eating the sterilized insects.

Miscellaneous controls

Many additional methods of pest control have been proposed but not yet developed to the point where they can be evaluated either with respect to benefits or hazards. Ultrasound has been proposed for use against insects and birds, but possible side effects are unknown.

In the more advanced orders of insects development of the adult reproductive stage requires the disappearance of a "juvenile hormone." Thus there is a potential for inhibiting insect reproduction by supplying this hormone artificially. The most obvious side effect is damage to beneficial insects such as bees, but there may be other effects.

Probably most plants and many animals have developed, in the course of evolution, specific chemicals that serve as pesticides or repellants. Some such as nicotine, pyrethrum, and rotenone are in use as pesticides and others such as the oil of poison ivy and the cantharidin of blister beetles are recognized as hazardous compounds. It seems at present impossible to predict what new and useful, or potentially destructive, discoveries may be forthcoming in this field.

Finally, there are possibilities for controlling pests by such devices as "trap crops" which are so attractive that the pests congregate where they can easily be controlled. For example, certain pests of cotton are preferentially attracted to alfalfa. An analogous case involving animals is the old practice of releasing guinea pigs in buildings infested with fleas for the purpose of collecting these parasitic insects. One can imagine that under some conditions trap crops might attract new pests as, for example, by bringing in undesirable weeds, but such possibilities seem not to have been investigated.

Appendix

INTERNATIONAL ASPECTS OF PEST CONTROL BY CHLORINATED HYDROCARBONS

The following breakdown on tonnage basis indicates the relative importance of insecticides in various crop groups in the world and the dependence on chlorinated hydrocarbons (aldrin, BHC/lindane, chlordane, DDT, dieldrin, endrin, heptachlor and toxaphene) is evident from second column (below). The source of this information is Shell International Chemical Co.'s worldwide usage survey for 1966.

Crop	Total insecticide usage, thousand tons	Percent chlorinated hydrocarbon insecticides in total
Cotton	60. 4	38
Rice	12. 0	57
All other cereals	7. 6	85
Vegetables	6. 8	46
Potatoes	2. 8	61
Sugar beet	2. 4	55
Sugarcane	2. 1	74
Tobacco	2. 0	67
Oilseeds	1. 9	77
Coffee	. 8	81
Tea	. 5	19
Sweet potatoes	. 2	92

Fruit including citrus, represent a relatively unimportant use of chlorinated hydrocarbons (CHI) on tonnage basis due to large volume of spray oils, however, chlorinated hydrocarbons on olives rate 55 percent and on vines 16 percent of total. DDT is used on fruit in Japan, South Africa, Mexico, Italy, United Kingdom, and Argentina, and BHC is used in Japan, India, and Algeria.

Each crop is discussed below in turn

Cotton.—Although only 38 percent of total, the chlorinated hydrocarbons are essential to this outlet in view of unrivaled cost/performance effectiveness and also the need to alternate with organo phosphorus products. Areas of major significance for use of CHI are: Mexico, Nicaragua, Egypt, Sudan, Brazil, Guatemala, Colombia, Australia, Turkey, Uganda, Ivory Coast and El Salvador for control of following pests: boll weevils, leafworm, lace bugs, stink bugs, cutworms, thrips, jassids, cotton stainers, tortrix larvae, lygus, mites, armyworms, white flies, aphids, pink bollworm, spiny bollworm and loopers, all of which are ubiquitous. Cyclodienes also play part in control of soil pests such as wireworms (Agriotes), white grubs, various coleopterious larvae, cutworms, and hylemya, either as seed dressings or preplanting soil treatments. Cotton losses due to insects compared with actual production (from Pflanzenschutz Nachrichten Bayer, 1967) are as follows:

Area	Actual production despite pests (thousand tons) (*A*)	Estimated actual losses due to insects (thousand tons) (*B*)	Percent total lost to insects
Central America	848	170	16. 7
South America	980	237	19. 5
Africa	1, 004	206	17. 0
Asia	2, 086	455	17. 9
Europe and Australia	175	30	14. 6
Total	5, 093	1, 098	17. 7

These losses occur not withstanding current insecticide use, which in cotton is essential for viable production. Figures for Europe include the eastern block. Letters *A* and *B* have same significance in all tables.

Rice.—Rice is very dependent upon BHC for control of various borers, armyworms, hoppers, beetles, but in addition endrin (stem borers, hoppers, rice bug, gall fly, jassid), DDT (cutworms, armyworms, flea beetles, loopers, paddy borer, paddy rice jassid, leaf hoppers), toxaphene (cutworms), and aldrin (termites, rice water weevil, gryllotalpa, tipula sp, paddy root weevil, paddy grasshopper) are of importance. Areas of major significance included Japan, India, Indonesia, Cambodia, Colombia, Venezuela, Taiwan, Brazil, and Spain.

Rice losses due to insects:

Area	Actual production despite pests (thousand tons) (A)	Estimated actual losses due to insects (thousand tons) (B)	Percent total lost to insects
Central America	921	41	4. 3
South America	7, 470	329	4. 2
Africa	5, 610	1, 217	17. 8
Asia (including Turkey)	154, 377	105, 700	40. 6
Europe	1, 585	37	2. 3
Total	169, 963	107, 324	38. 7

All other cereals.—(Maize, small grains, sorghum, etc.) These crops are highly dependent on CHI for insect control. Again BHC is most important for control of crickets, cutworms, wireworms, (where infestation not severe) myriads, lepidopterious larvae, and tipulidae in India, U.K., Mexico, Hautevolta/Niger, and Turkey. However, for soil pest control, e.g. wireworms, white grubs, crickets, cutworms, coleopterous root worms, dipterous root worms, of which first four are ubiquitous, there is really no substitute performance-wise for cyclodienes aldrin, heptachlor and dieldrin. Thus, these products are essential for these crops in France, Colombia, Chile, Mexico, Turkey, Spain, India, Argentina, East Africa, Japan, and Greece. Aldrin or dieldrin seed dressings give protection at extremely low cost and without risk of phytotoxicity to large areas of cereal crop and are valuable production tools especially in Argentina, Turkey, East Africa, Greece, India with particular reference to wheat.

Losses due to insects for wheat, oats, barley, rye:

Area	Actual production despite pests (thousand tons) (A)	Estimated actual losses due to insects (thousand tons) (B)	Percent total lost to insects
Central America	2,403	154	6.0
South America	15,977	867	5.1
Europe	129,049	4,940	3.7
Africa	9,645	1,437	13.0
Asia	42,753	2,978	6.5
Oceania	1,374	837	37.8
Total	201,201	11,213	

Losses due to insects for millet and sorghum:

Area	Actual production despite pests (thousand tons) (A)	Estimated actual losses due to insects (thousand tons) (B)	Percent total lost to insects
Central America			
South America	1,180	120	9.2
Europe	250	23	8.4
Africa	18,200	3,316	15.4
Asia	19,920	3,162	13.7
Oceania	219	22	9.1
Total	39,809	6,643	

Losses due to insects for maize:

Area	Actual production despite pests (thousand tons) (A)	Estimated actual losses due to insects (thousand tons) (B)	Percent total lost to insects
Central America	8, 450		
South America	19, 057	6, 353	25. 0
Europe	28, 324	1, 647	5. 5
Africa	14, 920	9, 503	38. 9
Asia	16, 510	2, 620	13. 7
Oceania	200	12	6. 0
Total	87, 461	20, 135	

Vegetables (excluding potatoes).—BHC is important in India, Japan, Mexico, Spain, but again where soil pests; e.g. agriotes, agrotis, hylemyia sp. gryllotolpa, and melolontha, need to be controlled cyclodienes are not replaceable on grounds of performance and lack of taint. Thus in crops, e.g. onions, tomatoes, chilies, and cabbage, where residue levels are acceptable, cyclodienes are important in Japan, Italy, Spain, France, and Portugal. DDT for control of cutworms, bollworms, white flies, jassids, fruit borers, webworms, cabbage moth, flea beetles, weevils, and armyworms is important in Mexico, Spain, Japan, India, Chile, U.K., Thailand and South Africa.

Vegetable losses due to insects:

Area	Actual production despite pests (thousand tons) (A)	Estimated actual losses due to insects (thousand tons) (B)	Percent total lost to insects
Central America	5, 000	639	11. 3
Europe	26, 645	866	3. 1
Africa	52, 994	7, 413	12. 3
Asia	61, 258	9, 209	13. 1
South America	35, 326	2, 634	6. 9
Oceania	836	104	11. 1
Total	182, 059	20, 865	

Potatoes including sweet potatoes.—Potatoes are heavily dependent on cyclodienes for control of wireworms and other soil pests and there are no suitable replacements. BHC is little used due to taint. Areas of importance for soil pest control include France, Spain, Brazil, Colombia, Peru, United Kingdom, Japan, Greece and Taiwan.

Foliage use of DDT is prevalent in Brazil, Colombia, Mexico, and Australia against thrips, tuber moth, armyworm, blister beetle, flea beetle and Colorado beetle.

Loss due to insects:

Area	Actual production despite pests (thousand tons) (A)	Estimated actual losses due to insects (thousand tons) (B)	Percent total lost to insects
Central America	537	108	16. 7
South America	7, 942	1, 922	19. 5
Europe	141, 543	9, 904	6. 5
Africa	1, 760	782	30. 8
Asia	11, 600	2, 035	14. 9
Oceania	720	74	9. 3
Total	164, 102	14, 825	

Sugar beet.—In view of nature of the crop cyclodienes (aldrin and heptachlor) again are very important for control of soil and ground surface pests such as agriotes, agrotis, gryllotolpa, melolontha, blaniulus guttulatus (millipedes), lixus, cleonus and pegomyia, particularly in Belgium, Italy, France, Spain, Greece, Chile and Turkey. Use of CHI seed dressings are essential when considering germination requirements of modern monogerm seeds, e.g. in U.K.; seed dressing in other areas, e.g. Turkey, are also important aspect where estimated 75 percent are treated this way. BHC use is important for cleonus, armyworm and beetles in Italy and Turkey and DDT is important in Turkey and Spain for similar pests.

Sugar beet losses due to insects:

Area	Actual production despite pests (thousand tons) (A)	Estimated actual losses due to insects (thousand tons) (B)	Percent total lost to insects
Central America			
South America	1, 034	229	18. 1
Europe	100, 080	6, 057	5. 7
Africa			
Asia	7, 440	3, 449	31. 7
Oceania			
Total	108, 554	9, 735	

Sugarcane.—BHC/lindane are dominant here and important in control of leaf hoppers, white flies, termites, soldier fly (Australia),[1] borers and cane beetles, in Mexico, Australia, India and Brazil. Endrin is important in certain areas; e.g. Ivory Coast, Taiwan and India, for borer control. Also, dieldrin is particularly relevant in South Africa where heteronychus licus is not controlled with any other product. Aldrin and heptachlor are important for soil pest control in India, Brazil, Mexico, Pakistan and Taiwan.

Sugarcane losses due to insects:

Area	Actual production despite pests (thousand tons) (A)	Estimated actual losses due to insects (thousand tons) (B)	Percent total lost to insects
Central America	101, 942	15, 446	13. 2
South America	117, 070	31, 358	21. 1
Europe	410	27	6. 2
Africa	30, 900	15, 450	33. 3
Asia	188, 120	134, 372	47. 7
Oceania	17, 670	2, 677	13. 2
Total	456, 112	199, 330	

Tobacco.—Foliage pests such as leaf worms, bollworms, thrips, hornworms, flea beetles, leaf miners, stink bugs, cutworms necessitate use of DDT in Mexico, Australia, South and East Africa. Likewise small quantity of BHC is used mainly in Mexico. Soil pests as previously listed are important in Japan, Italy, South Africa, Mexico, Colombia, Greece, and Spain, where cyclodienes are used instead of above two products, especially where wireworms are present.

Tobacco losses due to insects:

Area	Actual production despite pests (thousand tons) (A)	Estimated actual losses due to insects (thousand tons) (B)	Percent total lost to insects
Central America	247	41	14. 2
South America	244	77	24. 0
Europe	704	46	6. 1
Africa	260	57	18. 0
Asia	1, 460	218	13. 0
Oceania	18	4	18. 2
Total	2, 933	443	

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[1] Due to resistance can now be controlled only by dieldrin.

Oil Seeds.—These crops including sesame, soybeans, groundnuts, sunflower, etc. are again heavily dependent on CHI. BHC is important in India and Japan while DDT is used in Argentina, Brazil, India, Japan, Colombia, and Nicaragua. Toxaphene also is important in Colombia, France, Brazil, and Venezuela against lepidopterous larvae, earworms, armyworms, and loopers.

Losses due to insects:

Area	Actual production despite pests (thousand tons) (A)	Estimated actual losses due to insects (thousand tons) (B)	Percent total lost to insects
Central America	2, 854	255	8. 2
South America	5, 614	381	6. 4
Europe	5, 095	1, 378	21. 3
Africa	8, 785	2, 269	20. 5
Asia	19, 765	4, 971	20. 1
Oceania	366	91	19. 9
Total	42, 479	9, 345	

In addition to crop aspects there are certain other specific pest problems to be taken into account:

Locusts.—Total world annual value loss is estimated at $1 billion (Bayer 1967). In Asia and Africa more than 60 countries are liable to invasion by locust swarms. Medium-sized swarm may contain 3 billion locusts and consume 3,000 tons of food/day ("Pests and People," Shell). Several figures for value of crops destroyed in India, Kenya, Morocco, Libya, Sudan, Senegal, Ethiopia, India between 1928 to 1962 are available if required (Anti-Locust Research Centre London, Handbook). Recession of swarms was experienced from 1962 to 1968 but then a further outbreak started in 1969. However, this was checked and 1969 now is unlikely to become a plague year due to spray campaigns using BHC and dieldrin. The latter is still very important for control of hopper bands. Other insecticides including organophosphorus materials either are not so effective or more expensive.

Termites.—Total world annual value (excluding damage to woodwork, buildings, etc.) is $165 million (Bayer 1967). Annual expenditure in Australia for repairing damage done by termites is 3 million A. pounds (Pans February 1965, vol. 2). Termites in agriculture are covered where important in earlier sections. Nonagricultural use of aldrin/dieldrin is particularly relevant in building construction where many architects now specify dieldrin alone.

Yellow fever and malaria.—The main insecticide used against *Aedes aegypti* is DDT. One fifth of world's malaria has been eliminated by use of pesticides (Pans A, Vol. 9, No. 4, 1968). In India the number of malaria cases dropped from 100 million in 1933–35 to 150,000 in 1966, and loss to economy in same period reduced from 1.3 billion dollars to 2 million (World Health, April 1968). Dieldrin has played a part in this spectacular progress but DDT is still important in at least 24 countries for mosquito control and an estimated 16,000 tons are used annually. This is of particular importance in India, Mexico, Brazil, Venezuela, Columbia, Vietnam, Honduras, and Japan.

Chagas disease.—Residual house spraying with gamma BHC and dieldrin is successful in Central and South America. At moment, CHI are particularly important in Venezuela and Brazil.

Sleeping sickness.—This disease is transmitted by the Tsetse. According to "World Rev. Pest Control" (vol. 5, No. 1, 1966) between 1930 and 1946 half a million cases were reported and treated in Nigeria alone.

Four and one half million square miles of Africa is denied to cattle and horses with consequent inpoverishment of farming practice. DDT is used in Nigeria and Zambia but more effective control is obtained with dieldrin, which is widely used in Nigeria, Tanzania, Kenya, Uganda, and Zambia. Some endosolfan also used in this outlet. Use of organophosphorus insecticides is unsatisfactory.

River blindness (Onchocerciasis).—This is spread by simulium fly. It is of particular importance in West Africa where DDT is used in Nigeria, Ivory Coast, and Upper Volta.

Wood preservation.—In addition to public health, CHI are valuable in certain industrial uses, e.g. protection of wood products against borers etc., in wood-producing countries.

Tucura (Grasshopper).—This is a particular problem in Argentina, affecting 15 million hectares of which 7 million are natural grass and support average of 3 sheep or 0.8 cows/Ha. Eight grasshoppers per square metre eat equivalent to one animal/Ha. Thus, infested land in dry years is unable to support stock by mid summer and these have to be moved at great expense to tucara-free area or sold cheaply for slaughter. Prior to the use of dieldrin, annual losses of cattle products estimated at 90 million dollars despite extensive use of BHC. Dieldrin and heptachlor were banned February 1968 due to residue problem on USA imports, and subsequently four other products were approved: Sevin (expensive, difficult to apply), malathion (more expensive than dieldrin and average in performance) Diazinon (requires removal of animals from treated area), and Dibrom (requires special equipment). Sumithion trials were successful, but in practice control was poor, likewise control by malathion. Carbaryl, Diazinon, Azodrin, Gardona and Dimethoate gave poor or complete lack of control. At present there has been complete withdrawal of all approvals with resultant confusion. Next season Tucura infestation is expected to be severe due to lack of control this season, and if conditions are dry national disaster may result. Above case study clearly underlines the drastic effect that removal of the one essential agricultural weapon may have on an agricultural economy.

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CHAPTER 2

Contamination

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CONTAMINATION

SUMMARY AND CONCLUSIONS

The subgroup on contamination has examined the present status of knowledge on the dissemination of pesticides into the environment, the mechanisms and rates at which they accumulate in various elements of the environment, and methods by which pesticides might be controlled so that their presence in the environment would pose a minimal hazard to society consistent with the benefits to be obtained from their use.

The subgroup has examined: a) The air route by which pesticides are applied and distributed in the biosphere; b) the water route; c) the food route; d) soil contamination; e) household uses of pesticides; f) occupational exposures resulting from the manufacture and application of pesticides, and accidents that may occur in their use; g) alternatives to the use of persistent pesticides; h) the monitoring of pesticides in the environment; i) systems analysis of pesticides in the environment.

Much contamination and damage results from the indiscriminate, uncontrolled, unmonitored and excessive use of pesticides, often in situations where properly supervised application of pesticides would confine them to target areas and organisms and at the concentrations necessary for their beneficial use without damage to the environment. Research investigations, demonstrations, and monitored operations reveal that the careful application of many of the pesticides and the use of techniques presently available and being developed can be expected to reduce contamination of the environment to a small fraction of the current level without reducing effective control of the target organisms.

The present piecemeal involvement of various Federal agencies in pesticide control requires more than the existing type of coordination. As human health and welfare are the values of prime concern, the DHEW should provide a lead in the establishment of a mechanism for administering pesticide control programs.

Ad hoc studies of pesticides in the environment are not adequate to assess the inputs of pesticides to the biosphere, their degradation, translocation, movement and rates of accumulation. Monitoring is conducted by a large number of agencies, but in each instance the monitoring is related to a specific mission of the agency. Therefore, a single agency should take the initiative to insure the effective monitoring of the total environment, and the filling of gaps in data such as for oceans and ground water, as they are identified. A continuous systems analysis of pesticides in the environment needs to be conducted.

Aerial spraying should be confined to specific conditions of lapse and wind that will preclude drift. Regulations to limit aerial application to specific weather conditions would be helpful in providing guidance for regulatory programs. Increased engineering development effort is needed for the design of equipment for, and the adaptation of helicopters to the aerial spraying of pesticides.

The use of low volume concentrated sprays should be encouraged. Since this technique, if it is not properly controlled, can be more hazardous to workers, effective regulations must precede its increased use.

Increased information is needed on the degree of exposure of the general population to pesticides used for household, lawn, and gardening purposes. More effective means for regulation and control of pesticide use by the general public should be instituted, possibly by licensing of distribution outlets.

The use of indane and similarly toxic materials which act by evaporation must be discontinued where humans or foods are subject to exposure, such as in homes, restaurants, and schools.

There is a vastly increased need for the education of the general public in the management of pesticides and in the training of professional applicators. Public communications media, schools and universities all have important roles to play.

Labeling regulations must also be improved. Print should be enlarged and language should be made intelligible for the lay public. A need exists for nonlanguage, internationally intelligible insignia or markings that will advise the user of the degree of toxicity and persistance of the product, its method of application, and the target organisms.

More vigorous effort is needed to replace the persistent, toxic, and broad-spectrum pesticides with chemicals that are less persistent and more specific. Certain of the less-persistent pesticides, however, may be more toxic to humans and therefore effective regulation of their application is required to insure against injury to personnel.

Integrated control techniques for the control of select pests promises to effect a reduced usage of pesticides. Such alternative techniques should be more widely applied.

Licensing of commercial pesticide applicators, as well as other large-scale applicators of hazardous materials should be required.

Analytical methods, although extremely good, require further development. Need exists for standardizing or referencing additional techniques, even on an international basis. There is need for both less sophisticated techniques for field use as well as for automated techniques for wide-scale monitoring.

Standards for selected pesticides should be included in the Public Health Service "Drinking Water Standards". Although guidelines and criteria for some pesticides have been delineated, they have never been officially established.

Prior to application of pesticides to waters for the control of weeds, snails, mosquitoes, and in other aquatic uses, a careful analysis should be made of the proposed pesticide characteristics with respect to the uses of the target area. Special concern is indicated where domestic water supply is involved, or where food-chain concentration may occur.

Steps should be taken to prevent the simultaneous shipment of pesticides and foodstuffs within the same vehicle. Comprehensive regulations for pesticide transportation are required.

Safe methods of disposal of pesticides, their wastes, and containers are needed to prevent the contaimination of the environment and to protect individuals from contamination and accidents.

Intensified research and development is needed in the following areas, among others:

a. Prediction of the micrometeorological conditions suitable for aerial spraying.

b. Application of systems analysis to the pesticide-enviroment problem.

c. Pesticide chemodynamics, with emphasis on reservoirs of storage.

d. More intensive development of less-persistent pesticides with narrow spectra of toxicity.

e. Continuing development of spray devices with narrow spectra of droplet sizes.

f. Continuing development of alternatives to chemical control of pests.

g. Creation of more suitable materials for pesticide packaging and containers to facilitate safe transfer, handling use, and disposal.

h. Treatment processes for the elimination of pesticides from domestic water supplies as well as from wastewaters.

i. Immediate studies of the effects of pesticide residues on algal photosynthetic activity.

PESTICIDES AND PERSISTENCE

Any examination of environmental contamination by pesticides must include a consideration of persistence. Persistence in pesticides may be beneficial or harmful. Lasting residuals provide control of target organisms over longer periods of time and reduce needs for reapplication. However, lasting residuals may also affect nontarget flora and fauna for long periods of time.

Major classes of pesticides may be grouped as nonpersistent, moderately persistent, persistent, or permanent. Persistence times are those periods required for a 5- to 100-percent loss of the pesticides' activity under normal environmental conditions and rates of application (1). Accordingly, nonpersistent pesticides may be characterized as having persistence times of 1 to 12 weeks; moderately persistent pesticides, 1 to 18 months; and persistent pesticides, 2 to 5 years. Permanent pesticides are virtually permanent as they are not degraded. The overlaps and gaps simply illustrate the generality of the above definition of persistence time. Varying the environmental situation varies the persistence.

Important nonpersistent pesticides are organophosphorous compounds. They include malathion, methyl parathion, and parathion, which are widely used for the control of cotton and other pests. Malathion has also been developed for use as an undiluted ultra-low-volume spray. Another class of nonpersistent pesticides is the carbamates, which contain neither chlorine nor phosphorus, but are classified with most of the organophosphates in their low persistence. Carbaryl is the most used carbamate and was the third most used insecticide in the United States in 1964. Cotton, apples, and soybeans accounted for 62 percent of its total agricultural use. Its persistence is approximately that of malathion and parathion.

Most pesticides fall into the moderately persistent grouping. Nearly half of the total quantity of pesticide used in 1964 was used on corn, wheat, and cotton. 2,4–D and Atrazine, together, made up 54 percent of the total agricultural usage of herbicides in 1964. In 1967, herbicide sales in the United States exceeded insecticide sales for the first time.

The persistent group of pesticides include most of the chlorinated hydrocarbons. Of this group, DDT is still the insecticide most widely used worldwide although the overall demand for DDT, both in the United States and abroad, has declined. Most DDT exported is used in malaria-control operations. Within the United States, use will undoubtedly decline further, especially in view of mounting restrictions. The cyclodiene organochlorines include aldrin, dieldrin, endrin chlordane, heptachlor and toxaphene. Aldrin is currently used as a soil insecticide to control the corn rootworm. Dieldrin is used for the control of pests when a long-lasting residual effect is required. Toxaphene accounted for about 25 percent of all crop insecticides used in 1964, 69 percent having been applied to cotton.

The permanent group of pesticides are based on such toxic elements as mercury, arsenic, and lead. Once applied, these materials remain unless physically removed as by leaching with water. Since their water solubility is low, they tend to remain where applied.

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THE AIR ROUTE

The introduction of pesticides into the environment for the control of insects or unwanted plant growths is most generally accomplished by aerial or surface applications. In all such cases, air is the medium through which pesticides move to their intended targets. The characteristics of the air medium affects the efficiency of the application on the target and the dispersion of the pesticide outside the target area. A clear understanding of the ways in which air characteristics influence pesticide applications and of the presence and persistence of pesticides in air is necessary both for the efficacious application of pesticides and for the proper control of the possible harmful effects which these toxic chemicals may exert on nontarget organisms and the human populations.

In 1963 the State of California used about 20 percent of the total U.S. pesticide production (*1*). Approximately 80 percent of pesticides applied by commercial applicators were applied by aerial treatment and 20 percent by surface application. Problems associated with the application of pesticides by aircraft are therefore of primary importance. These considerations include the effect of the aircraft and its operation on the dispersal of the pesticide and the influence of meteorological conditions on aerial pesticide treatments. In addition, parameters such as wind drift, pesticide formulation and particle size, which are common to all pesticide applications, must be evaluated.

The application of pesticides by aircraft has increased over the last 30 years. Fixed-wing airplanes have been the dominant means of application but the use of helicopters has increased and is expected to increase further. Aircraft have been used to apply dusts, aerosols, granular materials and sprays.

Regardless of the pesticide formulation, the pattern of release from fixed-winged aircraft is from the craft into the air wake created by the wings. The wake carries the material outward to the wingtips, then drops it in a swath of about wingspan width. Two distinct vortices develop at the wingtips. The strong central propeller wash skews the wake to one side of the aircraft. The velocity of the particles is greater in the propeller wash than in the vortices. The wake which an aircraft produces is a function of the weight of the airplane and its load and the configuration of the wing and external applicating equipment. Therefore, the lighter and more aerodynamically clean the aircraft, the less turbulent is the wake and the more efficient and controllable the application.

The configuration of the particle movement behind a helicopter is similar to that of winged aircraft. Outer vortices develop but they are different in intensity due to the change in pitch of the rotor blades. The velocity of the particles is greater at the center of the rotor than in the wash created by the outer blades. Contrary to earlier opinion, the downwash of the helicopter rotor does not, at normal operating speeds, aid in the application of pesticides. In fact, above speeds of 15–25 mph forward speed, the helicopter does not exert any greater downwash than a winged aircraft. Only when the helicopter approaches hovering velocities does any significantly greater downwash occur. The spray pattern from helicopters is, however, better than that of an airplane due to the lack of a propeller wake.

The helicopter has other advantages over fixed-wing aircraft. It is highly maneuverable and is capable of flight at low levels and in mountainous terrain. The helicopter also has the ability to takeoff and land in small openings and it is not as dependent on airport facilities as fixed-wing craft. This reduces the turnaround time for reloading and may overcome the helicopter's two main disadvantages, its high cost and lower carrying capacity. On flat terrain or over forests where aircraft must fly faster than 45 mph and at heights of 50 feet or more above the treetops, slow, low flying fixed-wing aircraft are as efficient in application as helicopters and are less expensive to operate.

The spraying equipment used on fixed-wing aircraft and helicopters are similar in design. Dusts and granular materials are usually spread by means of a centrally located venturi device which mixes the material with air and discharges it into the trailing wake. Sprays are atomized by hydraulic atomizing nozzles and are distributed through a wing length boom. No benefit has been found by using longer-than-wing-length booms because the aircraft wake limits and controls the distribution of the particles. Booms are used on both fixed-wing aircraft and helicopters. Jet type nozzles aimed with the airstream have been used but produce a relatively coarse spray not suitable for all types of insecticide spraying. In most forest spraying, standard boom and nozzle spray systems have been used. They usually deliver 1 gal/acre at an atomization of 150 to 200 μ mass median diameter with the maximum drop size often exceeding 500 μ. Aircraft used for low-volume applications have been equipped with smaller nozzles or mini-spins calibrated to release 8 to 16 fl. oz./acre at a slighty finer atomization, usually 125 to 150 μ mass median diameter with a maximum drop size between 300 and 500 μ.

The atomization during low-volume spraying of mini-spin rotary units operating at three speeds and of three, small orifice, flat spray nozzles was investigated by Isler (2). An increase in the rpm of the mini-spin unit from 4000 to 8000 resulted in a decrease in the average mass median diameter (m.m.d.) of malathion drops from 184 to 120 μ. The best performing flat spray nozzle exhibited a range of m.m.d. of 112–158 with an average m.m.d. of 128, a value only slightly higher than that of the mini-spin at 8,000 rpm. The mini-spin, however, produced a narrower distribution of drop sizes than did the nozzle. At the low end of the spectrum, 25 percent of the spray volume of the mini-spin was in drops smaller than 90 μ, compared to 42 percent with the nozzle. The performance of the mini-spin, therefore, was more desirable than that of the nozzle because it resulted in fewer small drops which would be subject to drift and fewer large drops which would result in overdosing and inefficient spray application. This study demonstrates that the selection of the appropriate spray equipment is an important consideration in effectively applying agricultural chemicals.

Microclimatology effect.—The dispersal of particles during an aerial application is dependent on wind and thermal conditions at the site of application. At the time of application, calm wind conditions should prevail to minimize drift and allow for greater control of the pesticide application. Thermal conditions are also important since temperature gradients present in the area of application affect the movement and dispersion of the particles.

Akesson and Yates (*3*) state that the following measurements should be made for proper control of spraying: 1) The temperature gradient between 8 and 32 feet; 2) wind direction and velocity at 8 feet; and 3) the relative humidity at 8 feet. The temperature gradient between 8 and 32 feet will yield information as to the thermal condition present. There are two main types of thermal conditions. When the temperature decreases with elevation a *lapse* condition is said to exist. When the reverse is true and the temperature increases with height from the ground, the thermal condition termed *inversion* exists. Under various combinations of lapse conditions, the main particle movement is vertical whereas under conditions of inversion the main particle movement is in a lateral direction. The measurement of the temperature gradient between 8 and 32 feet indicates the degree of lapse or inversion conditions which exist. According to Akesson and Yates (*3*), a normal lapse condition would exist when a difference of about 0.1° F exists between the 8 and 32 foot elevations, with the lower elevation being warmer. (The commonly used dry-adiabatic lapse rate is 5.5° F/1,000 ft.) When the lower point becomes cooler, an inversion exists which puts a warmer air mass over cooler air and contains the spray particles in the area. Freshly irrigated or wet fields produce a low inversion as the wind carries the wet air over the crop.

The best weather conditions for application are under conditions of a normal lapse. With calm winds and an inversion condition between 8 feet and 32 feet (that is the temperature at 32 feet is +2° to +5° F. greater than at 8 feet), increased drift occurs because the "cap" of warm air prevents vertical movement and promotes horizontal or lateral movement. A lapse condition between 8 feet and 32 feet of −0.1° to −5° F. is usually indicative of turbulent weather. Large amounts of drift may then be expected to occur. The measurement of temperature gradients should therefore be considered as one of the best indicators of when and at what height sprays should be released at a particular spray site.

Pesticide formulation and particle size.—The formulation of pesticides, whether dusts, sprays, aerosols or granular material, and the size of the particles emitted, determines to a large extent: 1) The effect of microclimatic conditions on the spraying operations; 2) the potential exposure hazards, and 3) the persistence of the pesticide in the atmosphere.

The major climatic effect on spraying is that of wind. Dispersion of pesticide by drift as a result of windy conditions results in a loss in efficiency. If such losses occur, additional applications must be made which increases the cost as well as the hazard. More frequent applications also contribute to an increased insect resistence which, in turn, requires additional applications. Proper spraying procedures must therefore be developed. This is made difficult by the fact that many of the pesticides vary in their reaction to climatic conditions and the formulation itself affects their response.

Several studies which are illustrative of the variability in drift have been conducted. It has been found, for example, that the amount of drift occurring with aerial spraying is greater than that with surface spraying. Middleton (*1*) reported the results of the work of Wasserman *et al.* (*4*) who compared air concentrations in forests after surface and aerial applications of BHC and DDT. The results are as follows:

	Surface Treatment	Aerial Treatment
BHC	2.6–12.5 mg/m³ air	4.1–53.7 mg/m³ air.
DDT	4.6–25.5 mg/m³ air	18.9–170.9 mg/m³ air.

Thus, it is quite evident that the increased use of aerial applications is accompanied by an increased hazard from drift as well as a possible loss in the efficiency of the application.

Akesson *et al.* (*5*) measured the drift downwind after a DDT application of 1 lb./acre to 40 acres under both windy and calm weather conditions. They found the following:

	Concentration (p.p.m.) 100 feet downwind	Distance (ft.) 0.01 p.p.m. found downwind
Calm conditions	1. 5	8, 000
Windy conditions	8. 0	20, 000

The amount of drift, therefore, is highly dependent on wind conditions.

The amount of drift is also related to the size of the treated area and to the rate of application of the pesticide. The larger the area treated, the greater the number of swaths that must be made by the aircraft and the greater the potential for drift, but the lower the loss per unit area treated. Also, the greater the application rate, the greater the residue at given distance downwind.

Gerhardt and Witt (*6*) compared the drift of spray and dust formulations of DDT and found that under the same climatic conditions and at an application rate of 1 lb./acre, the spray resulted in a concentration of 0.1 p.p.m. DDT 2,640 feet downwind and the dust 1.4 p.p.m. at the same distance. This effect was due to the difference in particle size between the two formulations, the dust particles being smaller than the spray droplets.

Yeo (*7*) studied the effect of liquid particle size on drift and found that particles of less than 5 μ diameter exhibited little deposition and drifted in air currents for many miles. Particles of 10–50 μ diameter were deposited several miles from the source. Unless the wind was high, particles of 100 μ exhibited little drift hazard and when the particles were greater than 200 μ diameter, 80 percent were deposited within short distances downwind. Akesson and Yates (*3*) studied the effect of dust particle size on drift and found that when released 10 feet above the ground into a wind 3 m.p.h., 2 μ diameter particles traveled 21 miles, 10 μ particles 1 mile and 50 μ particles 200 feet.

Akesson and Yates (*3*) sprayed a field with toxaphene dust and spray and measured the drift of each as well as that of fine (95 to 150μ diameter) and medium size (150 to 300μ diameter) particles. They found that in all cases the ground fallout due to drift away from the target area was 4 to 10 times as high for dust than for sprays. Also, the ground residue from applications of fine drops was twice as much as medium drops at distances up to 1,000 feet. They also observed that in some cases, the amount of pesticide in the air downwind was 30 to 40 times that on the ground.

These studies indicate that as far as drift is concerned, the greatest potential nontarget contamination hazard occurs with smaller diameter particles. Since dusts contain a greater percentage of these smaller particles than do conventional liquid sprays, dusts constitute a greater drift hazard than sprays. Sprays, however, are not uniform in drop size and, with atomizers currently used, there is no way to eliminate the production of a wide distribution in drop sizes. Both large and small drop sizes will be produced. Moreover, the control of drop size in favor of the larger drops to reduce the drift potential results in a decrease in the efficiency of spraying since the efficiency is an inverse function of drop size. More large drops fall to the ground but the extent of coverage is less. Therefore a compromise in drop size must be reached between those sizes which minimize drift and a size which yields a high efficiency of application. Since a wide distribution in drop size is inevitable with commonly used spray equipment, and fine drops will inevitably be created, dependence upon large droplet size to control drift is not appropriate. The measurement of micrometeorological conditions, therefore, is of paramount importance in determining the safety of a proposed application.

This point is illustrated by a study conducted by Quinby and Doornink (8) in which the absence of wind and the presence of a thermal inversion caused problems even though calm weather prevailed at the time of the application. During an aerial application of TEPP (tetraethyl pyrophosphate), a thermal inversion and static air conditions, as well as topography which impeded the movement of the dust laden air and the presence of tall crops with dense foliage which constricted air movement, all combined to prevent the pesticide from dispersing with the result that persons and livestock near the dusting area were affected by the pesticide. The conditions of application were those that had been used successfully for 16 years prior to the date of the accidental poisoning. Therefore, for each individual application, appropriate measurements of microclimatology must be made and evaluated in order to insure that a safe and effective treatment is carried out, because as this work shows, while it is normally considered desirable to have little or no wind when spraying pesticides, such weather conditions may create a problem as serious as that caused by windy conditions.

Given good micrometeorological conditions, the size of pesticide particles or drops determines the efficiency of the application, i.e., the actual amount of pesticides which impinges on the target organisms. If the application efficiency is high, contamination by pesticides caused by particles which fall on nontarget organisms and/or foliage and soil, is minimized. If, in addition to more efficient application, pesticides less toxic to nontarget organisms and more selective to target organisms are used, then a reduction in environmental contamination will result.

In a study on the spray application for the spruce budworm, Mimel and Moore (*9*) attempted to resolve these problems. They felt that the spray operation should meet three conditions: (1) An insecticide which would be more toxic to the spruce budworm than to other organisms should be used; (2) the insecticide should be amendable to breakdown in the forest ecosystem and thus not be accumulated in plant and animal systems; and (3) the pesticide should be directed to the target insect with a higher degree of efficiency than to other organisms.

Zectran, a carbamate insecticide, was selected for use because it demonstrated a high degree of selectivity for the budworm. It is more toxic to the budworm than DDT and has a relatively high acute oral toxicity, which are the main potential hazards in field use. Also, Zectran and its metabolites are readily broken down by sunlight and in plant and animal systems. The problem of directing the spray to the spruce budworm with greater efficiency than to any other organism was then studied.

It has been demonstrated that most forest spraying has been conducted with particles of 150μ mass median diameter, i.e., 50 percent of the particles are of drop sizes greater than 150μ diameter. This was true even though earlier work indicated that drop sizes of more than 100μ penetrated vegetation only slightly or not at all. Moore made a study of the drop sizes which would most efficiently penetrate the forest canopy. Fine fluorescent particles were suspended in the spray. These particles distributed themselves according to the spray volume in any given drop. By counting the number of flourescent particles remaining after a given drop had evaporated, it was possible to determine the approximate original size of that drop.

When the size and number of spray drops impinging on each spruce budworm larvae was studied, it was found that no significant number of drops larger than 100μ diameter and only a small number of droplets between 50 and 100μ diameter reached the target insects. Only drops below 50μ diameter reached the budworm larvae with any degree of efficiency. This is significant when it is considered that 95 percent of the spray volume of spray systems normally used in forest insect control are larger than 50μ diameter. Thus, a great proportion of this spray fails to reach the target insect and becomes a major source of environmental contamination. This study resulted in the development of a spray system which eliminated all drops above 120μ diameter.

Potential exposure hazards.—Pesticides vary in their toxicity to humans. The potential exposure of a person to a given pesticide often is determined by the formulation and particle size of the material. Thus, a compound which may not be considered toxic to humans but that has been formulated in a way which promotes easy entry into the body, may be potentially more harmful than a more toxic pesticide with a more difficult entry path. Particle size is an important factor.

The three main modes of entry of pesticides into the body are by: (1) inhalation; (2) skin absorption; and (3) ingestion. Hayes (*10*) has rated the three major pesticide formulations (gases, dust, and sprays) according to their potential for entry by the above routes. For respiratory intake, the most hazardous formulation is a gas. Next are dusts and the least dangerous are sprays. Thus, the danger from this route of entry decreases with increasing particle size. The maximum danger due to skin absorbtion is from sprays and liquids, with dust posing the lowest threat. Hayes noted that some gasses can pass through the skin.

The effect of particle size on exposure was studied by Wolfe *et al.* (*11*). The dermal and respiratory exposures to parathion of workers using both conventional dilute spray machines and low-volume concentrate sprays were compared. The particle size of the concentrate spray is smaller (20 μ to 100 μ diameter) than that of the conventional spray machine (over 150 μ diameter). The potential dermal and respiratory exposures associated with the two spraying methods were found to be as follows:

	Concentrate spraying technique	Conventional dilute spraying method
Dermal exposure	27.9 mg./hr	19.4 mg./hr.
Respiratory exposure	0.055 mg./hr	0.02 mg./hr.

Most of the difference in the dermal exposure was attributed to a greater hand exposure with the concentrate machine. The difference in respiratory exposure was considered to be caused by the smaller drop size produced by the concentrate spraying method.

The potential danger of pesticides to handlers and operators is well known. The literature is replete with accidents which have occurred with adverse, sometimes lethal, consequences. There is less information available, however, concerning the pesticide exposure to the general population. Such information is vital if the proper assessment of the risk to the general population is to be determined.

Risebrough *et al.* (*12*) measured the concentration of pesticides over Barbados, an area remote from the agricultural use of pesticides, and found that the total amount of pesticides in the air ranged from less than 13×10^{-6} nanograms per cubic meter of air to 380×10^{-6} ng/m^3. By contrast, the air at La Jolla, Calif., an area adjacent to agricultural areas where pesticides are used, contained an average of 7.0×10^{-2} ng/m^3. Thus, the authors concluded that pesticides are universally present in air and that their distribution from application sites is dependent on the prevailing patterns of wind circulation and the rates of fallout.

In 1965, the occurrence of a dust storm of unusual intensity allowed a study of the transport of pesticides over long distances and the subsequent precipitation to earth by rainfall to be conducted (*13*). The dust storms were spawned on January 25 in the southern high plain's of Texas. The dust-bearing air mass gradually moved eastward, spreading out in a north-south direction and narrowing in an east-west direction due to airflow patterns within the air mass, and by January 26, parts of the dust had reached Cincinnati, Ohio. Dust was collected in Cincinnati and analyzed for its pesticide content. The major pesticide components of the dust were DDT and chlordane with concentrations of 0.6 and 0.5 p.p.m. respectively based on the air-dried weight of the dust. DDE and Ronnel followed closely in concentration. These four pesticides, together with three other pesticides, heptachlor epoxide, 2,4,5-T and dieldrin, which occurred in lesser amounts, made up the major portion of the pesticide content of the dust.

This study gives evidence that pesticides may be transported over long distances when attached to dust particles in the air. These particles may be washed out of the air by rainfall and deposited on the ground. Evidence is also provided that the pesticides can survive degradation by photochemical reactions in the atmosphere and can be deposited over land surfaces remote from their point of application.

A study was carried out by Stanley (*14*) with the express purpose of determining the atmospheric contamination by pesticides in the United States. Sampling sites were established at nine locations: Baltimore, Md., Buffalo, N.Y., Dothan, Ala., Fresno, Calif., Iowa City, Iowa, Orlando, Fla., Riverside, Calif., Salt Lake City, Utah, and Stoneville, Miss. Both urban and rural sampling sites were chosen.

Chlorinated hydrocarbons such as DDT, BHC, DDE, lindane, dieldrin, and aldrin, and organophosphorus pesticides such as parathion and malathion were sought. Only DDT was found at all localities. The DDT concentrations were highest in the agricultural areas of the South. Pesticides were found from the lowest level of detection (0.1 ng/m^3 air) to as high as 1560 ng/m^3 p-p′ DDT, 2520 ng/m^3 toxaphene and 465 ng/m^3 parathion.

The highest pesticide levels were found when spraying was reported to have occurred just prior to the sampling. There was no correlation between pesticide levels and rainfall. In most cases, when the pesticide levels decreased after a rainfall had been reported, it was also noted that spraying activities had also ceased because of the rain. The decrease in pesticide levels could therefore have been due to the cessation in spraying operations.

The kinds and levels of pesticides found varied with the agricultural activity in a given area. The pesticide levels varied from season to season according to the chemicals used and crops grown during each season. Most pesticides were present in the atmosphere as particulates. There was little correlation between the pesticide level and the time of day. These concentrations, even the highest levels measured, are below those encountered by the general population from other sources. For example, Durham *et al.* (*15*) analyzed 12 restaurant and 17 household meals and found that, based on the food in the meals analyzed, the mean daily intake of DDT was 1.99×10^{-1} mg. If a person inhales 7000 1/day of air containing 1560 ng/m^3 DDT, he will inhale 1.092×10^{-2} mg/day.

It is obvious from this study that broader sampling programs must be undertaken in order to establish the pesticide levels to which the general population is exposed. In the study just mentioned, both urban and rural sampling sites were chosen, but in different localities. It might be more meaningful to take both urban and rural samples at the same locality rather than to try to compare pesticide levels of rural Mississippi with urban California or New York. In order to study the pesticide levels in the United States, perhaps one section of the country should be concentrated on at a time. An area in which a large amount of pesticides is being used, for example the Southeast, could be selected for study. Within this area, urban and rural samples would be collected in order to establish the amounts of pesticides transported from the rural spraying areas to the urban center. In this way, urban exposure could be related to the spraying activities in the surrounding rural area.

Persistence of pesticides in air.—The presence of pesticides in air is a function of their chemical nature, physical state, method of application, and atmospheric conditions. The persistence of pesticides in the air—i.e., the length of time the particles remain in the air—is also a function of these factors. Pesticides may be lost from the air by several means. Gravitational fallout and washout by rain are perhaps the two major factors which cause pesticide removal from the air. Exposure of pesticides in air to sunlight and reactive compounds results in degradation or modification of the compounds by photolysis and catalysis, but the amount of pesticides removed from the air by these processes is unknown.

The persistence of pesticides in the air is also a function of their volatility, particularly with spray operations. Spray particles decrease in size as they fall, due to evaporation and codistillation. If they evaporate completely before they fall to the ground, dust particles remain and are more liable to drift.

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THE WATER ROUTE

The introduction of pesticides into the water environment can occur even before the raindrop comes into contact with a pesticide-treated surface. Rainwater was collected continuously over a 12-month period in the British Isles at seven widely distributed sites. At all sites and throughout the year, rainwater contained alpha-BHC, gamma-BHC, dieldrin, pp'DDT, pp'DDE, and pp'TDE. The concentrations were extremely minute, being of the order of parts/10^{11} or parts/10^{12} (*1*).

The major pathway of pesticides into the water environment occurs through direct application to surface waters and surface run off. The major recipients of surface run off are streams, lakes, and coastal waters.

Ground waters.—Some pesticides ultimately find their way into ground waters, but the contribution of pesticides to a surface water such as a perennial stream from a ground water source is—like rainfall—minute. Many variables are responsible for the small concentrations of pesticides usually found in ground waters. These include the type of pesticide, its solubility, formulation, and mode of application, the soil types, climate, season, amount of soil organic matter, microbiological populations, and yet other factors. Organochlorines, for example, have been demonstrated to move only short distances in soils via the leaching process (*2*). Another study concluded that it was most unlikely that parathion could contaminate underground water supplies by leaching, under normal rainfall conditions (*3*). Russian workers found that lindane and heptachlor do not contaminate ground waters when used in quantities encountered in agricultural practice, but concluded that to avoid the possibility of contamination, these organochlorines should not be used oftener than once every 2 or 3 years (*4*). Working with dieldrin, Eye (*5*) concluded that it cannot be transported through soils into subsurface waters in significant amounts by infiltrating water because of the extremely long time—several hundred years—necessary to transport the dieldrin in solution through the top 12 inches of soil. However, ground waters must be vigilantly protected since, once polluted, the contamination can persist for long periods of time. That they can become polluted is documented in the Montebello, Calif., and Colorado arsenal incidents, both of which involved 2,4-D (*6*).

The Environmental Control Administration's Bureau of Water Hygiene recently (summer 1969) queried several States about incidents of well water contamination by pesticides. The reply from Illinois, succinctly characterizes the response from all the States: "* * * To our knowledge we have never had a public well water supply contaminated with pesticides. Through the years we have had several instances of private wells being contaminated by and large through accidental spills and back siphonage directly into the well. A typical example is a well in Adams County where a farmer was making up a tank of pesticide. He left a power pump in operation while he went into his farm home for lunch. While he was there the motor stopped and the total content of the tank was back siphoned into the well. We have rarely had an example of a well being contaminated by underground infiltration. The few instances where we have had such condition have been the result of the application of termite control materials in a trench immediately adjacent to a water supply well."

From Wisconsin it is reported that it took several months to remove the residual taste from the water of wells accidently contaminated by 2,4–D during which time cattle would not drink the water. An analysis of a private well water in Ohio showed 0.0033 mg. of chlordane per liter but no information was available on the route of entry. Another well contained 2,4–D in a concentration of 0.0155 mg./*l.* which gained entry through run-off from an area sprayed by the State Highway Department.

The Indiana response stated: "We have learned to expect requests for help in three or four cases of chlordane in wells every spring or early summer when exterminators treat for termites. The pesticide normally associated with termite control is chlordane although one of the labels sent to us this spring included one percent of an extract of Lindane." One of the property owners whose well was contaminated during termite extermination reported that his water still tasted of chlordane a year later.

In connection with the State's Ground Water Quality Control Act in Michigan, consideration was given "to the possibility of pollution of the soil or ground water from the mixing and preparation of spray materials, fertilizers, or chemicals," and that a 150-foot isolation distance is specified between a well and the preparation or storage area for such chemicals.

Preliminary results from a Bureau of Water Hygiene survey of private well water supplies in one State indicate that pesticide pollution of ground waters is more wide-spread than commonly realized, although the concentrations are very low. At the time of writing only nine samples had been analyzed, but all nine showed DDT. In addition, three contained endrin, two contained chlordane, and one each contained dieldrin and heptachlor. Although all concentrations were less than one microgram per liter, the possibility of concentrations increasing with time must be considered.

Surface runoff.—The 1967 production of pesticides of 1050×10^6 lbs. if applied continually to the annual U.S. runoff (1.16×10^6 million gallons per day) would result in a concentration of 0.3 mg./*l*(7). Although this exercise is purely academic (most of the production is exported and most of that which is used is degraded or tightly bound to soils) it does point out the extensive use of pesticides by our society.

Because of the tight binding characteristics of pesticide residues to soil particles, it is suggested that the general pollution of water by pesticides occurs through the transport of soil particles to which the residues are attached (8). Problems of sampling and analysis preclude the use of systems designed to give continuous read-out of pesticide concentrations in water. It is a common finding in water analysis that fish may contain pesticides in excess of Federal tolerances, yet water samples from the same environment may fail to reveal the presence of residues at levels of 1×10^{-3} mg./*l*. (9). It is not surprising, therefore, that shellfish—known for their accumulating characteristics—have been proposed as monitors of water quality for pesticides. Laboratory experiments showed that oysters and other bivalves remove chlorinated hydrocarbon pesticides from experimentally contaminated water and store them in tissues. In clean water, the residues are flushed out at uniform rates. Using oysters, Butler (10) conducted a 3-year, 170-station study of estuaries that demonstrated that pesticide pollution is primarily the result of agricultural practices in the associated river basins, and that in specific locations, industrial or municipal wastes and noxious insect control programs are major sources of pollution.

The Public Health Service Laboratory at Dauphin Island, Ala., clearly demonstrated the seasonal variation in pesticides content of oysters in Mobile Bay. Peak total DDT levels occurred in late winter and early spring months, coinciding generally with the maximum fresh water inflows and minimum salinities of the Bay waters (11).

Nicholson (*12*) notes the close affinity of pesticides to soil particles and suggests that the soil-loss equation used in guiding conservation farm planning be applied to the prediction and control of pesticide pollution associated with rural run off. The affinity may also be a basis for water pollution control recommendations based on geographic zones.

Lakes.—Geologically speaking, lakes are usually transitory bodies. They are born, they live and age, and they die. Their aging process is termed eutrophication. That man's influence grossly accelerates the process is well documented; witness Lake Erie. Lakes and oceans are veritable sinks for man's wastes. But lakes are infinitely more susceptible to man's negligence because of their much smaller size. Even larger lakes, however, are not immune as witness the Lake Michigan Coho salmon and Lake Erie eutrophication problems. To illustrate the concentrations of pesticides present in Lake Michigan water, the results of grab samples taken from the two intakes to the Chicago filtration plant during April 1969, showed the following:

Pesticide	Concentration—ppm	
	Plant 1	Plant 2
Lindane	0.020×10^{-3}	0.010×10^{-3}
Heptachlor epoxide	0.049×10^{-3}	0.019×10^{-3}
Aldrin	0.019×10^{-3}	----------------
DDT	0.058×10^{-3}	0.034×10^{-3}
pp′ DDT	0.122×10^{-3}	0.029×10^{-3}

A limited number of surface samples collected by the Bureau of Commercial Fisheries, Ann Arbor Biological Laboratory, from Lake Michigan in 1968 showed the following:

DDT	2.0–2.8 $\text{ppm} \times 10^{-6}$
DDD	0.3–0.5 $\text{ppm} \times 10^{-6}$
DDE	0.8–1.4 $\text{ppm} \times 10^{-6}$

Lakes with waters of lesser amounts of dissolved minerals are more biologically sparse than more eutrophic lakes. An interesting experiment was performed in Oregon wherein two mountain lakes were treated with the organochlorine toxaphene. One lake was deep and biologically sparse, the other was shallow and rich in aquatic life. The deeper lake could not be restocked with trout for 6 years because of toxic quantities of toxaphene remaining in the water. Trout were restocked in the shallow lake within one year. Explanations offered for the slow recovery of the deeper lake include thermal stratification due to depth, a slow rate of dilution from small tributaries, and markedly less plant and animal life (*13*).

An important aspect—perhaps the most important aspect—of chemodynamics of a lake and which is fundamental to the life of the lake as well as to the effects of pesticides therein involves the sediment-water-chemical interchange. These relationships are well suited to modern systems analysis and mathematical modeling and deserve increased study.

The possible buildup of pesticides in the bottom sediments of lakes, with the result that they become, in effect, reservoirs of pesticide residues, has received little study. If this phenomenon occurs in lakes which undergo thermal stratification, the pesticides could theoretically be resuspended with the sediments during the fall overturn and present aquatic organisms with additional exposure to higher concentrations than would be expected. Such periodic exposure could lead to an increased biological accumulation in the food chain.

Most of the studies that have described the fate of pesticides introduced into aquatic systems have found that the decrease in water pesticide concentration which occurs with elapsed time after the application is accompanied by an increase in the concentrations of pesticide found in the bottom sediment and/or the organisms. For example, Bridges *et al.* (*14*) applied 0.02 p.p.m. DDT to a farm pond and observed that the surface water concentration decreased from 0.08 p.p.m. one-half hour after treatment to zero in 4 weeks. The concentration of DDT in the bottom mud prior to treatment was found to be 0.08 p.p.m. One-half hour after treatment, DDT concentration in the mud was 0.21 p.p.m., and after 8 weeks the mud contained 0.19 p.p.m. At this time vegetation samples contained 5.1 p.p.m. DDT. Twelve months after treatment, the vegetation had returned to pretreatment levels. Fish accumulated 3 to 4 p.p.m. DDT and its metabolites within 1 month after the treatment. The levels declined to 2 to 3 p.p.m. 15 months later. Cope (*15*) reports on a study in which 20 p.p.m. DDT were added to a microcosm system containing water, mud, vegetation, sunfish and snails. The water concentration decreased to 0.42 p.p.b. in 14 days but the mud concentration had increased to 6 p.p.b. and the vegetation to 15.6 p.p.m. The fish contained 1.0 p.p.m. DDT after 2 weeks and the snails 160 p.p.b.

Undoubtedly not all pesticides exhibit the same phenomenon. For example, the water in a pond contaminated by endrin as a result of aerial spraying of an adjacent field at the rate of 6 oz. of active ingredient per acre was found to contain 0.04 p.p.m. endrin 4 days after treatment (*16*). The water concentration declined for 21 days after which no more endrin was detected in the water. Endrin concentrations in the mud after 45 days were found to be 0.35 p.p.m. after which time the endrin disappeared.

Although some pesticides may not exhibit long-term accumulations in aquatic systems, the possibility exists that reservoirs of pesticides became available for recycling back into biotic systems. Therefore, studies on the rates of pesticide interchange across mud-water interfaces and between vegetation and water and the magnitudes of the pesticide levels involved should be conducted.

Estuarine waters

Pesticides are a matter of serious concern to the shellfishing and finfishing industry as well as to sports fishing enthusiasts. Water quality standards for pesticides as a means of protecting fish resources appears to be of value for shellfish but the evidence is not so clear for game fish. The studies of Mobile Bay oysters by the Public Health Service (*11*) demonstrates the ability of shellfish to cleanse themselves of pesticides. Butler (*17*) reported an ability of shellfish to cleanse themselves of 0.03 to 1.5 p.p.m. of various organochlorines in a 10–20 day period when placed in clean water. A similar ability for finfish has not yet been demonstrated.

Although estuarine areas wherein oysters are found would appear highly vulnerable to pesticide contamination, continuing surveillance demonstrates that the need for standards is not urgent (*18*). However, a surveillance program is highly recommended (*19*).

The importance of estuarine areas as the nursery areas of the world's marine food production is discussed by Butler (*20*). He states that industrial and domestic pollution of many estuaries has already so degraded the environment that only the most tolerant of organisms can persist, and that the remaining uncontaminated estuaries are of major importance. As pristine qualities cannot be restored to degraded estuarine environments, consideration must be directed toward the provision of estuarine hatcheries similar to those used for fresh waters.

The inhibition of marine algal photosynthesis by DDT was investigated by Wurster (*21*). Four algal species, including a diatom from Long Island Sound, a coccolithophore and a green alga from the Sargasso Sea and a neritic dinoflagellate, were tested, as well as a marine phytoplankton community, by exposing them to DDT and measuring their photosynthesis as indicated by the fixation of C^{14}. In all cases an increase in the concentration of DDT from 1 p.p.b. to 100 p.p.b. produced a decrease in photosynthesis from the photosynthetic level of unexposed control algae to approximately 10–40 percent of the control photosynthesis. At a constant 10 p.p.b. DDT, the inhibition of photosynthesis decreased with increasing cell concentration due, according to the author, to a decrease in the amount of DDT in solution per cell. Wurster states that his laboratory experiment demonstrated that reduced phytoplankton populations in nature might be caused by DDT. DDT might also encourage blooms of some algal species after the selective stress of the chemical has produced a decrease in other, less tolerant, community phytoplankters. Continued increases of concentration of persistent pesticides in the marine environment might then have long-term impact on total photosynthetic activity, perhaps inducing a change in oxygen and carbon dioxide partial pressures in the atmosphere.

Potable water supply

The Public Health Service Advisory Committee on Use of the PHS Drinking Water Standards recommended limits for select pesticides (*22*). These limits were derived for the Advisory Committee by an expert group of toxicologists as being safe if ingested over extensive periods. The limits for four of the pesticides (aldrin, heptachlor, chlordane, and parathion) were set at even lower values because of their organoleptic properties. The pesticides and their limiting concentrations are:

Pesticide	*Concentration, mg./l.*
Aldrin	0.017
Chlordane	0.003
DDT	0.042
Dieldrin	0.017
Endrin	0.001
Heptachlor	0.018
Heptachlor epoxide	0.018
Lindane	0.056
Methoxychlor	0.035
Organic phosphates plus carbamates	0.1 [1]
Toxaphene	0.005
2,4=D+2,4,5=T+2,4,5=TP	0.1

[1] As parathion in cholinesterase inhibition. It may be necessary to resort to even lower concentrations for some compounds or mixtures.

It should be noted that these criteria have never been officially adopted by the Public Health Service in their Drinking Water Standards although the operating program, the Bureau of Water Hygiene, utilizes the criteria as guidelines.

Water quality criteria related to recreation as used by the Public Health Service contain no limits for specific pesticides. The need for their control is emphasized in terms of satisfactory conditions being preserved through watershed management: *viz.*, evaluation of potential health hazards through consideration of the toxicity, persistence, and exposure hazards of any pesticides to be used (*23*).

Water is a vehicle for transporting wastes and most waste-treatment plants use biological processes. The effects of pesticides on these biological stabilization processes are so far not of great importance at the concentrations usually encountered (*24*). However, effects can be disastrous at the high concentrations that might result from spills or accidents.

The removal of pesticides by standard water treatment processes has been found to vary with the individual pesticide and the concentrations encountered. In general, it is much more difficult to remove the low levels of pesticides that occur through continuous contamination from runoff, etc., than to reduce the high levels which result from direct application and accidents.

Cohen *et al.* (*25*) studied the effectiveness of standard water treatment processes for removing rotenone and toxaphene and concluded that the single most effective treatment was the use of activated carbon.

Rotenone and toxaphene at concentrations of 100 p.p.b. were reduced to 3 p.p.b. and 7 p.p.b., respectively, with 5–6.6 p.p.b. carbon. Alum coagulation failed to remove either pesticide. Chlorine and chlorine dioxide were ineffective in reducing the concentration of toxaphene but did oxidize rotenone. The concentrations required, however, were so high that the residual oxidants had to be removed by dechlorination.

Aly and Faust (*26*) (*27*) found that activated carbon was the most effective method for removing the herbicide 2,4-D from water. At an initial concentration of 1 p.p.m., 13.6–16.2 p.p.m. carbon was required to reduce the 2,4-D concentration to 0.1 p.p.m. The authors found that precipitation with limestone products did not remove 2,4-D from the water because of the high solubility of calcium and magnesium salts. Neither oxidation by chlorination or potassium permanganate nor coagulation with ferrous sulfate and alum at concentrations of 100 p.p.m. removed 2,4-D from water.

The removal of low concentrations of toxaphene and BHC by carbon adsorption at a municipal water treatment plant was studied by Nicholson *et al.* (*28*). During a 4-year period, the concentrations of toxaphene in untreated water was measured at 7 to 270×10^{-6} p.p.m. while the concentration of toxaphene in treated water during the same period ranged from 5 to 410×10^{-6} p.p.m. The concentrations of BHC ranged from 7 to $1{,}004 \times 10^{-6}$ p.p.m. in untreated water and from 9 to 640×10^{-6} p.p.m. in treated water. It was concluded that the treatment process used was ineffective in removing the low concentrations of toxaphene and BHC.

The use of other techniques for the removal of low levels of pesticides from water has not received extensive study and requires further evaluation. Such methods involve the use of other adsorptive media, ion exchange resins, selective membranes and pH adjustment and require that the chemical characteristics of the specific pesticide that is to be removed be taken into account.

The available data suggest that periodic occurrences of high pesticide levels in water may be reduced to acceptable concentrations by water treatment practices and that adsorption by activated carbon is the most effective such treatment. Low level, long-term contamination, however, is much more difficult to remove and evidence indicates that current water treatment methods do not eliminate this possible source of human exposure.

The Report of the National Technical Advisory Committee to the Secretary of the Interior (*22*) on water quality criteria for most industrial uses does not indicate pesticide contamination to be a major concern. However, water required for food and kindred products and for the leather industries is specified to be of drinking water quality.

Pesticides can have detrimental effects on irrigation water quality, directly or indirectly. The phenoxy acid herbicides (2,4-D has been mentioned in the Montebello, Calif., and Colorado arsenal incidents) are suspected contaminants of irrigation water. Bruns (*29*) found that when 2,4-D at 6 lbs. per acre in irrigation water was applied to red Mexican beans in the seedling stage, the root systems were severely damaged and the yield was reduced by 40 percent. When applications at the same level were made at the bloom stage, the loss rate dropped to 29 percent.

Tentative guidelines have been suggested for certain herbicides and for specific crops (*22*). An example is provided by:

Herbicide	Type of application	Reference crop	Concentration mg./l. [1]
Acrolein	Furrow	beans	60
		corn	60
		cotton	80
		soy beans	20
		sugar beets	60
	Sprinkler	corn	60
		soy beans	15
		sugar beets	15

[1] Crop injury threshold in irrigation waters.

For most pesticides, however, too little is known about their ultimate fate and their influence on irrigated agriculture. However, thus far evidence does not indicate that under normal use insecticide contamination of irrigation water is detrimental to plant growth or accumulates in or on irrigated plants to toxic concentrations.

Pesticide-laden irrigation waters may be a source of contamination to nearby streams as a result of flooding and/or runoff. Miller *et al.* (*30*) observed the movement of parathion from cranberry bogs treated at the rate of 1 lb. per acre into a nearby irrigation ditch and drainage canal. A parathion concentration of 30 p.p.b. was found in the drainage canal immediately after spraying as a result of water seeping through the floodgate. After 24 hours, the concentrations had decreased to 3×10^{-3} p.p.m. At this time 2,4-D was also detected 50 and 150 yards down the drainage canal indicating movement of the pesticide from the point of application. The use of a water tight floodgate would minimize seepage but the overflow of the bog and irrigation ditch as a result of a heavy rain would remain as a potential source of contamination of waters and soils in nontarget areas.

In a study of the fate of aldrin in rice paddies, it was found that 2 days after seeding with treated seeds at an application rate of 6 oz. per acre, the water in the paddy contained 1.6 p.p.b. aldrin plus deildrin (*31*). After the third and last drainage of the paddy, 14 weeks after seeding, the water contained 0.07 p.p.b. aldrin plus dieldrin. Up to 0.027 p.p.b. aldrin plus dieldrin was found in the ditches into which the paddies were drained after 14 weeks and the small stream which received this water contained up to 0.44 p.p.b. The river into which the stream flowed contained as much as 0.023 p.p.b. aldrin plus dieldrin. Concentrations in the river decreased to below 0.006 p.p.b. after several more weeks.

In the same study, 7 days after the treatment of a cotton field with 0.4 lb. per acre of endrin, a concentration of 0.66 p.p.b. endrin was found in the runoff following a 1.15-inch rain. Used irrigation water contained 0.11 p.p.b. endrin 3 days after spraying. Prior to irrigation, the water had contained 0.08 p.p.b. endrin. These data indicate that irrigation water applied to fields following the application of pesticides may be contaminated after use. The degree of contamination depends on the time interval between the pesticide application and the irrigation water use, as the water concentrations decrease with elapsed time. The concentrations of pesticide in runoff after heavy rains, following pesticide application may be significant. Godsil and Johnson (*32*) studied the pesticides in water used to irrigate the areas surrounding the Tule Lake and Lower Klamath Lake Wildlife Refuges located in northern California. Approximately 156,000 acres of land lie upstream from these lakes and extensive use of pesticides is made in the area. Water is used and reused throughout the irrigation system and samples were taken at a point where the water had been recycled an estimated 5.2 times. Endrin, because of its abundant usage in the area as well as its high degree of persistence, was found in the irrigation water in greater amounts than any other chemical. The concentrations of endrin in the irrigation water increased during the growing seasons and decreased to the limits of sensitivity between seasons. The endrin concentrations of submerged plants, clams and fish followed a similar pattern. A maximum of 0.10 p.p.b. endrin in the water and 198 p.p.b. in tui chubs was found during one growing season. Thus, the pesticide concentration of irrigation water and aquatic biota were directly associated with the agricultural activities in the area. Between growing seasons, both the water and the organisms returned to low levels of contamination. No mortalities were observed as a result of this contamination. Although these results indicate that short-term pesticide contamination does not result in a permanent residual concentration of pesticides in aquatic organisms, the possible long-term hazards may only be postulated.

Direct spraying.—Pesticides are applied directly to waters for the control of mosquitoes, obnoxious or undesirable weeds, and snails. In order to minimize the potential harm of such applications, both to nontarget biota and to human consumers of water, an analysis of the proposed pesticide's characteristics with respect to uses of the target area must be conducted prior to its application. An example of a successful large-scale direct application of a pesticide to water which serves both as a recreational facility and as a water supply is provided by Smith and Isom (*33*) who describe the application of 2,4-D for the control of Eurasian watermilfoil growth in TVA reservoirs. Very stringent controls were required in order to preserve the aquatic components of the system as well as to minimize the contamination of drinking water taken from the impoundments.

Eight hundred and eighty-eight tons of a 20 percent 2,4-D butoxy ethanol ester granular herbicide were applied to 8,000 acres in seven reservoirs at rates varying from 40 to 100 pounds of 2,4-D acid equivalent per acre. Prior to the application, laboratory experiments determined that concentrations of 2,4-D much in excess of the concentrations that would be encountered in the field, were not completely toxic to mosquito larvae. This indicated that the aquatic fauna would not be adversely affected by the concentrations resulting from the application.

Following the application of 2,4-D, raw water samples were analyzed by activated carbon absorption at nine water treatment plants. At eight of the plants, concentrations in the raw water of less than 1 p.p.b. 2,4-D were found, and at the ninth, 1 to 2 p.p.b. were detected. Treated water from the ninth treatment plant contained less than 1 p.p.b. 2,4-D. Significant mud concentrations were observed following treatment, however, and these residues remained high for a considerable period of time. In the Watts Bar Reservoir, for example, 58.8 p.p.m. 2,4-D butoxy ethanol ester was found 10 months after treatment.

In the reservoirs examined, no significant change was observed between pre- and post-treatment numbers of burrowing mayflies, indicating that the benthic invertebrate populations had not been harmed. The elimination of the watermilfoil did, however, result in a significant loss of aquatic insects which utilized submerged vegetation as a habitat. Although little uptake of 2,4-D by fish was observed, instances of the accumulation of the herbicide by freshwater mussels were observed.

The large-scale application of a herbicide for the control of nuisance aquatic plant growth with a minimum of harm to the aquatic ecosystem is demonstrated by this study. The results could not have been obtained had not the herbicide used been carefully selected.

In another investigation, preliminary studies failed to reveal all the hazards involved with the proposed direct application of a pesticide to a lake for the control of a nuisance organism. The insecticide DDD was applied directly to Clear Lake in California for gnat control in 1949, 1954, and 1957 by pouring a liquid concentrate of DDD from barges (*34*). The resulting water concentrations were estimated to be

14 p.p.b. in 1949, and 20 p.p.b. in 1954 and 1957. It was determined before the applications that DDD at these concentrations was not toxic to fish and other aquatic organisms. Dead western grebes, however, were found in 1954, 1955, and 1957 in areas surrounding the lake. An analysis of the fatty tissue of the birds which died in 1957 indicated that DDD was present at a concentration of 1600 p.p.m. Subsequent DDD analyses were made on members of the fish populations and it was found that all fish contained DDD, and that carnivorous fish contained more than plankton-feeding fish. It was concluded from this study that the grebe losses, occurring after the DDD applications, were caused by chronic DDD poisoning resulting from eating DDD-contaminated fish. Therefore, studies prior to the application, which indicated that the DDD concentrations used were not toxic on a short-term basis, did not take into account the biological concentration of the pesticide through the food chain.

Mosquito control.—Current mosquito control practices utilize both larvacide and adulticide techniques. The Southern operations—including the States of Alabama, Florida, Georgia, Louisiana, Mississippi, South Carolina, and Texas—rely on light oils applied with spreading agents in their larvaciding operations. Aircraft application is used for large areas at the rate of 5 gallons per acre. Paris green pellets are also applied by some districts, and, again, sometimes the aerial route is used. Resistance is the primary reason that organochlorines and organophosphates are not used.

The Southern operations also rely extensively upon engineering and drainage techniques. These are not too amenable to Western activities, however, where irrigation is a prime cause of mosquito burdens. These areas have also met with extensive resistance problems and have gone through chemicals such as DDT and Abate. Last year, Dursban was utilized in large-scale tests because it appeared that this chemical would be a more economical choice than oils or Paris green pellets.

Adulticiding operations utilize ground equipment such as the new Leco ULV (ultra-low-volume) and chemicals such as malathion and Naled. Aircraft—fixed-wing and helicopters—have been used to apply Baytex, and organophosphate. ULV and fogging operations are often conducted in populated areas.

Municipal and industrial waste discharges.—As with many other industrial processes, the wastes of the pesticide manufacturing and formulating industries usually may not safely be introduced directly into receiving bodies of water. The treatment required to reduce the toxicity of the wastes to levels which will not endanger aquatic systems varies with the components of the wastes themselves. Some compounds are amenable to treatment by chemical and biological processes while others are more resistant to conventional treatment. Settling basins are often used to allow time for waste decomposition and for impoundment during periods of low flow. Solid and heavy liquid wastes may be incinerated effectively, often in combination with the scrubbing of effluent stack gasses for removal of harmful vapors. Adequate incinerator operation is essential. The deep well disposal of wastes may be practical in some instances but, because of the risks inherent in this disposal technique, a thorough evaluation of the geological characteristics of the area and the nature of the waste involved is required in each specific instance before this method is employed.

Water concentrations.—As part of the National Water Monitoring Network, samples of a water-suspended sediment mixture from 11 streams in the Western part of the United States were analyzed monthly for 12 different pesticides beginning in October 1965 (*35*). The compounds for which analyses were made included aldrin, DDD, DDE, DDT, dieldrin, endrin, heptachlor, heptachlor epoxide lindane, 2,4-D, 2,4,5-T and silvex. All insecticides were found at one time or another but not at all stations. The amounts observed were small, ranging from less than p.p.t. (parts per trillion) lindane, to 110 p.p.t. DDT. Insecticide concentrations of 5 p.p.t. or more were found in slightly more than 50 percent of all positive samples. No herbicide was found at any time at any station.

Although no definite seasonal pattern could be noted in pesticide occurrence, positive results were more frequently found in February, March, April, and May. Lindane was the most frequently found insecticide and occurred in 46 of the total 165 positive results. The most infrequently occurring pesticide was aldrin which was observed only four times at all stations. The most frequent occurrence of pesticides was at the Rio Grande River station below Anzalduas Dam, Tex., where 20 percent of the total positive results were observed. The least number of positive results was observed at the Snake and Columbia River stations each of which recorded only seven pesticide occurrences. Since the amounts of pesticide applied in the various areas could not be ascertained, no relationship could be made between residues in the water and the agricultural use of pesticides.

Studies of the chlorinated hydrocarbon pesticide content of sediments in water from the lower Mississippi River and its tributaries were conducted in 1964, 1966, and 1967 to determine the extent of possible sources of agricultural pesticides in the streams of the Delta (*36*). There were two areas of significant pesticide contamination, one in Memphis, Tenn., and the other in Mississippi. Both were in association with chlorinated hydrocarbon pesticide manufacturing plants. Pesticide residues were detected from both agricultural and nonagricultural areas. There was, however, no evidence of a general build up of chlorinated hydrocarbons in the sediments of these streams from the agricultural use of pesticides. DDT analogs and associated metabolites were found in some of the tributary streams where no known formulators were located. These data indicate that the large amount of chlorinated hydrocarbon pesticides applied to crops in the Mississippi River Delta have not created widespread contamination of the water and sediments in the area.

As a part of a nationwide program to determine the levels of cholorinated hydrocarbon pesticides in estuaries, nine California estuaries were sampled during 1966 and 1967 (*37*). Shellfish were used as sampling organisms because of their ability to concentrate low pesticide levels in the marine environment. Among the pesticides found were lindane, heptachlor, aldrin, heptachlor epoxide, DDT, dieldrin, and endrin. Based on the wet weight of homogenized oyster, mussel, and clam tissue, DDT, DDD, DDE, dieldrin, and endrin were found in estuaries in concentrations from 10 to 3,600 p.p.b. High levels of DDT, DDD, DDE were observed from offshore exposure, as the king crab contained 2,739 p.p.b., and the ova from a cape salmon 668 p.p.b. The pesticide levels of shellfish in estuaries receiving runoff from agricultural and urban areas were found to be as high as 11,000 p.p.b. The pesticide residues in estuaries geographically isolated from agricultural areas seldom exceeded 100 p.p.b.

From 1958 to 1965, samples taken at more than 100 stations in the major river basins of the United States were analyzed by carbon adsorption for their chlorinated hydrocarbon content (*38*). Dieldrin, endrin, and DDT concentrations as high as 0.100 p.p.b., 0.116 p.p.b., and 0.148 p.p.b., respectively, were found at various sampling stations.

The results of this study indicate that the most widely found chlorinated hydrocarbon pesticide was dieldrin which occurred most frequently in all river basins. Endrin was found occassionally in the early years of the survey with increases in incidence occurring in 1962 and 1963. The maximum frequency of occurrence of endrin was found in 1964, particularly in the lower Mississippi River, after which endrin levels decreased. DDT and its associated compounds were found regularly from the beginning of the study with a slightly increasing trend in evidence.

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The Food Route

CROP APPLICATION

There is a tendency toward the overuse of pesticides both in agricultural as well as in domestic activities. In large-scale operations the dusting or spraying operation is frequently conducted with the avowed purpose of serving as a prevention of blight or insect infestation in order to assure against potential loss. In some cases an assessment of the actual existing infestation or evidence to indicate the exact dosage required would lead to considerably smaller amounts of pesticides being used.

Excessive household use of pesticides occurs because of the complete lack of a scientific approach to their employment. In cases where large scale applications are made by plane, commercial spraying or fogging equipment, attention must be given to problems of drift associated with wind and air currents. Depending upon the degree of infestation and the nature of the meteorological conditions, occasions have arisen where the felt need for pesticide application did not allow the postponement of the spraying operation until more favorable meteorological conditions existed. In such cases, drift has been experienced to a distance as great as 100 miles.

Systemic versus surface-type pesticides.—Some research has been conducted on plant systemic pesticides which allow the pesticide to be picked up by the roots of the plant and carried through the plant system. To date, while some applications of this type have been successful, the overall prospects for wide-scale adoption do not appear to be very promising. In addition to the potential of reducing the waste of the more conventional types of application such an approach would increase contamination of the soil. Questions have also been raised in regard to the use of plant systemic pesticides in relation to the possibility of the contamination of the fruit and plant which are subsequently used for human or animal feed.

Pesticides from soil to plants.—Crops may absorb pesticides from soil previously treated. In areas where short-term crops are raised, such as in truck farming where the crops have relatively short growing periods and where repeated applications of pesticides may be used for each crop, there is evidence that a considerable amount of pesticide residue remains in the soil and is picked up by the plant and deposited in varying amounts in the food product.

For example, Lichtenstein *et al.* (*1*) found that cucumbers grown on soil treated once a year for 5 years with aldrin, dieldrin, and heptachlor at the rate of 5 pounds per inch-acre contained up to 0.011, 0.102, and 0.23 p.p.m., respectively. Similar residues were found in both the upper and lower halves of the cucumbers. The pesticide residues had penetrated the root system and were translocated to the fruit. In the same study, it was found that alfalfa grown on soil containing 0.5 p.p.m. per inch-acre of aldrin or heptachlor would contain approximately 0.005 p.p.m. of aldrin and dieldrin or 0.015 p.p.m. of heptachlor and heptachlor epoxide. Similarly, Wood *et al.* (*2*) found that alfalfa grown on fields treated 4 and 5 years earlier with dieldrin at 5 pounds/acre contained as much as 0.03–0.04 p.p.m. dieldrin. The residues of corn grown on the same fields did not differ greatly from those of the alfalfa.

Bruce *et al.* (*3*) studied the relationship between the pesticide residues in several crop seeds, the soil concentration and fat content of the seeds. Their results are as follows:

	Seed	Soil residue p.p.m.	Seed residue p.p.m.
Heptachlor and heptachlor epoxide	Corn	0. 5–4. 0	0. 002–0. 01
	Barley	0. 5–4. 0	0. 002–0. 02
	Oats	0. 5–4. 0	0. 008–0. 08
Aldrin and dieldrin	Corn	0. 35–3. 5	0. 002–0. 008
	Barley	0. 35–3. 5	0. 006–0. 015
	Oats	0. 35–3. 5	0. 007–0. 08

Seeds with high fat content such as soybeans and peanuts contained greater pesticide residues than did seeds of lower fat content.

Nash (*4*) grew wheat in soils treated with DDT and dieldrin at 0.5, 2 and 10 p.p.m. Dieldrin residues in the wheat seedlings increased in direct proportion to the soil application rate. DDT residues also occurred in the seedlings but to a lesser extent. After 3 weeks, dieldrin seedling residues were approximately 18 percent of the soil concentration and DDT residues approximately 3 to 10 percent. The transfer of pesticides contained in a soil to crops via the root system can therefore be a significant source of food contamination.

Metabolism of pesticides by the plant.—Studies of the ability of plants to metabolize pesticides which have been applied either to the surface of the plants or which may be found in the soil have indicated that in some cases this process can serve as a means of reducing the persistence of the pesticides. For example, the destructive hydrolysis of organophosphate insecticides on plant surfaces and within plant cells (especially for systemic insecticides) allow such strong pesticides as TEPP, Phosdrin and Mevinphos to be used safely on edible fruits and vegetables within a day or two of harvest. Persistent chlorinated hydrocarbon insecticides, on the other hand, do not undergo degradative reactions in such short time periods.

In other cases, however, more harmful and/or persistent compounds may be reduced by metabolic processes. It is well known that within plant cells aldrin may be converted to epoxide dieldrin and heptachlor to heptachlor epoxide. The vapor pressure of the epoxide compounds are lower than that of the parent chemicals with the result that the half-life values of the pesticides increase from about 1 day to 7–8 days.

The effect of plant characteristics on residue.—A number of plant characteristics require additional application of pesticides at various stages of production. These include growth rate of the plant, foliage density, shape of the plant, surface characteristics of the leaves, stems and branches, problems of the enzyme systems of the plant, and the metabolic rates of the plant. The amount of residue which actually comes in contact with the plant and remains effective depends to a great extent on all of these factors.

Time factor.—The pesticide residue carried to human food may depend on the period of time between the application of the pesticide to the crop and the harvest and processing of the crop. The intensity of the application, growth, dilution, and degradability are coupled with the time and method of processing. All of these factors must be considered in the assessment of the amount of pesticide which reaches the ultimate consumer.

Mann and Chopra (*5*), for example, found that carbaryl residues on cabbage and eggplants decreased exponentially from the day of application. Their results, after spraying the plants at three different rates every 3 three weeks for 9 weeks, are as follows:

	Initial residues—p.p.m.		
	0.55 pound/acre	1.1 pound/acre	2.2 pound/acre
Cabbage (0 day)	14. 80	23. 83	33. 86
Cabbage, washed (0 day)	0. 97	1. 34	1. 25
Eggplant (1 day)	8. 33	12. 22	16. 86

	7-day residues—p.p.m.		
	0.55 pound/acre	1.1 pound/acre	2.2 pound/acre
Cabbage	2. 50	3. 94	5. 13
Cabbage, washed	1. 44	2. 17	2. 94
Eggplant	3. 05	4. 31	5. 40

The rate constant was 0.17–0.28 per day for both vegetables and the half-life values for cabbage and eggplant were 3 and 3.2 days respectively. These results indicate that ample time must be allowed for pesticide die off before processing, even though carbaryl and other pesticides do exhibit a rapid residue loss rate. It is therefore important that the rate of residue loss is known by the farmer and food processor, in order that pesticide application and crop harvesting and processing may be coordinated. These results also indicate the process of pesticide dissipation may be speeded up by such procedures as washing.

The use of the crop.—The route of pesticides to man may depend upon the use of the crop in question. For fiber products, the residue on the crop may cause less concern, except in cases of those persistent pesticides which may cause dermatitis or which are toxic by skin absorption.

Some food products which are subjected to a series of pesticide applications are consumed by the public without extensive processing. In other cases, the processing of the food may remove a portion of the pesticide residue while in other cases the food processing method may serve to concentrate and accentuate the pesticide problem in the food product.

Surveys of food in the markets of the nation indicate varying amounts of pesticide residues in many commercial foods. The total dietary exposure varies from one part of the country to the other and depends upon the dietary habits of the individual or family. Few, if any, foods are completely free from some degree of pesticide residue.

The dietary intake for pesticide chemicals in the United States from June 1966 to April 1968, is reported by Duggan and Lipscomb (*6*). The market diet studied was that of 16- to 19-year-old males, a group that consumes greater quantities and kinds of food than any other. The study was conducted in five geographical regions of the United States and the foods were prepared for consumption before analysis.

During the period from June 1966 through April 1967 (referred to as the 1967 period), residues of 15 chlorinated hydrocarbons, 6 organophosphates, 4 herbicides, and 2 carbamates were detected. Similarly, between June 1967 and April 1968 (designated as the 1968 study period), residues of 18 chlorinated hydrocarbon chemicals, 8 organophosphates, 5 herbicides, and 1 carbamate were found.

The following table reports the daily intake and percentage of total daily dietary intake of each of the four organic pesticide classes.

	Total intake mg/day		Percent total daily intake	
	1967	1968	1967	1968
Chlorinated hydrocarbons	0. 081	0. 072	69. 8	85. 8
Organophosphates	. 018	. 006	15. 5	5. 5
Herbicides	. 004	. 004	3. 4	5. 0
Carbamates	. 013	. 002	11. 3	3. 7
Total			100. 0	100. 0

Chlorinated hydrocarbon pesticides.—The high proportion of chlorinated hydrocarbon residues in the diet is expected because of their greater persistence and wide use. The daily intake of chlorinated hydrocarbons has been relatively consistent since 1964. DDT and its analogs comprised approximately two-thirds of the chlorinated hydrocarbon residues found during the 1967 and 1968 periods. DDT alone accounted for approximately one-fourth of the total intake of chlorinated hydrocarbons. The major sources of the chlorinated hydrocarbons were those food classes representing products of animal origin, namely, "dairy products and meat, fish and poultry." These foods were the source of approximately one-half of the total intake of chlorinated hydrocarbon residues. In view of the fact that these products received little direct application of pesticide chemicals, their presence must be due to indirect and environmental sources. Grain, fruits, and garden fruits combined to account for about 40 percent of this class of pesticides. The dietary intake from the remaining seven food classes studied: Potatoes; leafy vegetables; legume vegetables; root vegetables; oils, fats, and shortening; sugars and adjuncts; and beverages, accounted for about 10 percent of the chlorinated hydrocarbon residues.

Dieldrin, lindane and heptachlor followed DDT and its analogs in magnitude of dietary intake. The incidence and amounts of other chlorinated hydrocarbons detected in the study were too low to be of any dietary significance.

Organophosphate insecticides.—Approximately one-third of the organophosphate insecticides were found in grains and cereals. Malathion accounted for 80 percent of the calculated daily intake of organophosphates. The average daily intake for this 2-year period was 0.009 mg./day. The incidence and intake of the remaining seven organophosphorous insecticides detected were too low to be considered as regular components of the diet.

Herbicides.—About 50 percent of the herbicide residues detected during the 1967 and 1968 periods were in foods of animal origin, that is, dairy products and meat, fish and poultry. This is again idicative of environmental contamination since herbicides are not used directly on these products. The two most frequently found herbicides were 2,4-D and PCP.

Carbamate insecticides.—The incidence and amounts of carbamates were very low in both study periods. The insecticide carbaryl was found in four composite samples in 1967 but was not found at all in 1968. For 1967 its calculated daily intake was 0.006 mg./day. This group of chemicals did not occur at sufficiently high levels of frequencies to be considered as contributors to the daily intake of pesticide chemicals.

Dithiocarbamate residues were found in a few samples in both study periods. Evidently this class of insecticide decomposes sufficiently during harvesting and processing so that it is not regularly found in ready-to-eat food.

Inorganic residues.—Inorganic bromides were found in approximately 80 percent of all samples examined during the 1967 and 1968 periods. Bromide residues were found in all food classes, but the highest residues were found in the grain and cereal class. The average daily intake for the 2-year study period was approximately 24 mg.

Arsenic residues were detected in 10 percent of the composite samples examined in 1967 and in 18 percent of the samples analyzed in 1968. The daily intake of arsenic, calculated as As_2O_3, was 0.33 mg. for 1967 and 0.137 mg. for 1968.

When the dietary intake of pesticide chemicals is compared with the acceptable daily intake proposed by the Food and Agricultural Organization of the United Nations and the World Health Organization Expert Committee on Pesticide Residues, it is found that no acceptable daily intake value was exceeded by food residues during the 1965–1968 period. The daily dietary intake for practically all pesticides was at least one order of magnitude (1/10) or more below that considered safe by the FAO–WHO reports. The average combined level of aldrin and dieldrin, however, was approximately equal to the acceptable daily intake values. This is significant because, except for DDT and its analogs, dieldrin is the pesticide most frequently found in food. The following table compares the FAO–WHO acceptable daily intake values with the dietary intake of several pesticides during the 1965–1968 period.

	FAO-WHO acceptable daily intake [1]	Expended dietary intake [1]			
		1965	1966	1967	1968
Aldrin and dieldrin	0. 0001	0. 00009	0. 00013	0. 00006	0. 00006
Carbaryl	. 02	. 0021	. 0005	. 0001	----------
DDT, DDE, TDE	. 01	. 0009	. 0010	. 0008	. 0007
Lindane	. 0125	. 00007	. 00006	. 00007	. 00004
Heptachlor and heptachlor epoxide	. 0005	. 000033	. 00005	. 000021	. 000031
Malathion	. 02	----------	. 0001	. 0002	. 00004
Parathion	. 005	----------	. 00001	. 00001	. 000001
All chlorinated hydrocarbons		. 0012	. 0016	. 0012	. 0011
All organophosphates			. 00014	. 00025	. 00007
All herbicides		. 00012	. 00022	. 00005	. 00006

[1] (mg/kg. body wt./day).

In summary, the kinds, frequency and levels of chlorinated hydrocarbon pesticides found in the total diet samples during 1967 and 1968 do not differ significantly from those found from 1964 and 1966.

Chlorinated hydrocarbon residues were commonly found in all diet samples and in all food classes except beverages. The incidence of organophosphorus pesticides increased during the 4-year study period. Malathion was the major contributor of this group to dietary intake. The incidence and levels of herbicides have remained low throughout the 4-year study. No herbicide residues were found in legume and root vegetables and garden fruits. The incidence of carbaryl and carbamate chemicals were too low for these pesticides to be considered as regular constituents of the diet.

Foods of animal origin were the main source of chlorinated hydrocarbon residues in the diet. These foods comprised about one-fourth of the diet used in the study. These foods were the source of about one-half of the intake of total chlorinated hydrocarbon residues and DDT compounds and were the source of even a greater proportion of heptachlor epoxide, BHC and dieldrin. Since the residue levels of aldrin and dieldrin are about the same as the acceptable levels of these compounds, it is possible that the acceptable daily intake may be exceeded under certain dietary patterns. Reductions in residue levels in foods of animal intake would be the most effective means of reducing the dietary intake of pesticides, particularly the more toxic pesticides.

The exposure of the general population, therefore the impact of pesticides on the health of man, is much greater through the food route than either air or water. Although "market basket" studies indicate that the exposure of the population to pesticides through food over the last few years has generally not increased, present levels of intake, particularly for aldrin and dieldrin, may be some cause for concern. At any rate, so far as the health of the general population is concerned, the greatest emphasis in pesticide control should be on reducing the concentration in foods. Methods for such reduction are available and are being applied on an increasing scale.

Animal feed.—Market basket surveys indicate that foods of animal origin contain relatively high levels of pesticide residues. As meat and dairy products are not directly treated with pesticides, the consumption of contaminated feed is probably responsible for the subsequent buildup of pesticides in animal tissues. The wide variety of components such as fish, soybeans, and cottonseed contained in animal feed increases the potential contamination of the feed by pesticides.

The length of time that the residues remain at high levels in animal tissue varies with the pesticide and the concentrations consumed. Often the tissue residues decrease after the cattle stop eating contaminated feed. If the cattle were killed and prepared for human consumption while still eating contaminated feed or immediately after they had stopped eating such feed, however, significant residue levels in the meat might be found if they were originally high. The build-up of pesticide residues in cattle tissue was studied by Rusoff *et al* (*7*) who measured the residues of heptachlor epoxide in the fatty tissue of cattle which grazed on pasture treated with 0.25 lbs./acre heptachlor. The cattle were allowed to begin grazing on the field at intervals of 1, 8, 15, 29, and 43 days following the application of the pesticide. The cattle which began grazing 1 day after treatment contained 2.5 p.p.m. heptachlor epoxide in the fat. No pesticide was found in the cow which began grazing 43 days after treatment indicating that the pesticide level in the forage had declined. The fatty tissue residues decreased with increased elapsed time before grazing began. Several cows were allowed to begin grazing on a field during an application of 0.25 lbs./acre heptachlor. The average fat content in these cows was found to contain 3.45 p.p.m. heptachlor epoxide after 29–30 days. The concentration decreased with increasing time from the application. Less than 1 p.p.b. heptachlor epoxide residue was found in the raw and cooked meat of cattle which had grazed for 125 days. This study indicates that although pesticide residues do build up in tissues as a result of eating contaminated feed, the residues are reduced after such feeding is terminated.

Claborn *et al.*, (*8*) found that after feeding toxaphene to cattle over an 8-week period at rates of 60, 100, and 140 p.p.m., fat residues were 8.4, 14.3, and 24.3 p.p.b. respectively. Claborn *et al*, (*9*) also found that no residues of Sevin were found in the tissues of cattle, sheep, goats, and hogs after they had been sprayed four times in 2 weeks with a 1 percent suspension. One goat contained Sevin in the fat of the brain. The tissues of Hereford steers fed 50 and 20 p.p.m. Sevin for 27 days also contained no residue.

The excretion of pesticides in milk after cattle have consumed forage previously treated with pesticides can also be a problem. Cows fed DDT in their diet at rate of 0.5, 1.0, 2.0, 3.0, and 5.0 p.p.m. DDT in alfalfa exhibited DDT in their milk at all feeding levels except 0.5 p.p.m. (*10*). At 1 p.p.m., residues of 0.01 to 0.03 p.p.m. were consistently present in the milk after 19 days. As the DDT feed levels increased, the DDT contamination in milk increased. At a feeding rate of 5 p.p.m. DDT, significant DDT levels appeared in the milk from a Guernsey cow. The milk concentration during the feeding period ranged from 0.16 to 0.32 p.p.m. Cows were also fed toxaphene at rates of 2.5, 5, 10, 15, and 20 p.p.m. for 77 days (*11*). The milk concentrations increased with the amounts in the food. The residue levels in the milk plateaued after the 28th day except for the 2.5 p.p.m. level at which a plateau was reached after the 9th day. Plateau levels in the milk at the 2.5, 5, 10, 15, and 20 p.p.m. toxaphene feeding level were 0.043, 0.076, 0.100, 0.173, and 0.179 p.p.m. respectively. A rapid decline in pesticide milk residues was observed after the feeding of toxaphene was terminated and, 14 days after the termination, almost all the cows produced milk with nondetectable amounts of toxaphene. Thus it appears that milk residues as well as tissue residues decrease after termination of the feeding on contaminated forage. Not all pesticides produce residues in milk. Loeffler *et al.*, (*12*) found that Guthion did not produce detectable residues in milk at feeding levels of 4.2 to 33.3 p.p.m. Guthion metabolites were detected, however, but disappeared within 3 days after treatment was discontinued.

The spraying of cattle with insecticides for insect control may produce milk and tissue residues. Claborn *et al.*, (*8*) sprayed cows with toxaphene and strobane in a 0.5-percent emulsion, a 0.5-percent suspension, and a 2-percent oil spray. These concentrations are entomologically effective. The emulsion and suspension were used twice at 3-week intervals and the oil spray twice daily for 21 days. The maximum residues in milk (0.61–0.71 p.p.m.) occurred 1 or 2 days after the first spraying with the emulsion and suspension and then decreased to low values at 21 days (0.05–0.12 p.p.m.). There was no significant difference in residue levels between the two formulations or two insecticides. The residues of both toxaphene and strobane reached the maximum (0.28–0.41 p.p.m.) about the third day of oil spraying then remained at approximately the same level for 18 days. Since no residues are permitted in milk, these insecticides are not recommended for use in the control of cattle insects. Thus, animals which are fed on feeds containing pesticide residues or which are sprayed with pesticide for insect control may serve as an additional source of pesticides to the consuming public.

Moubry *et al.*, (*13*) studied the decline of chlorinated hydrocarbon pesticides in the milk of cows. After pesticide residues were detected in the milk of several herds, all efforts were made to remove the cows from exposure to pesticides by removing the cows from contaminated food and/or dermal spraying for insect control. After removal from the pesticide sources the residues eventually declined to a point below actionable levels. Dieldrin had the longest retention time in milk, approximately 100 days. DDT and its analogs, BHC, lindane, endrin, and methoxychlor follow in that order. The amount of DDT, DDD, DDE residues in milk varied in relation to each other depending upon whether the animal exposure was by ingestion or dermal application. This knowledge is useful when determining the source of pesticide exposure.

Direct exposure.—Various foods receive a direct exposure to pesticides during the growing season and, depending upon the methods of processing, may contribute considerable pesticide to the diet of the food customer. The problem to the consumer in this situation depends upon the degradability of the pesticide, the time factor between the application and the final processing, the time factor in shipping and the amount of pesticide applied. The degree of penetration of the pesticide into the food material is also of concern with certain types of food.

Koivistoinen *et al.* (*14*) studied the fate of malathion residues from post-harvest treatments during food processing of gooseberries, plums, tomatoes, apples, and stringbeans. The approximate residue reduction produced by the various processing techniques were: Canning, 50 percent or more; processes with a cooking period of 15 to 20 minutes, 30 to 50 percent; juice making by pressing or steaming, 70 to 90 percent; drying at 75° C. for 1 to 2 days, 90 to 100 percent; and freezing, 40 to 50 percent. Losses of from 0 to 79 percent were obtained by washing the fruits in running water for 1 minute. Virtually no malathion was lost when the processed materials were stored at 4° C. for up to 8 months.

The removal of DDT, malathion, and carbaryl from tomatoes by commercial and home preparative methods was studied by Farrow *et al.*, (*15*). Commercial canning and juicing operations removed practically all DDT, malathion, and carbaryl residues. All but trace amounts of DDT and malathion were removed by the home canning of whole tomatoes and tomato juice. Approximately 92 percent of the carbaryl residue was removed by canning whole tomatoes and 77 percent removed by the home canning of tomato juice. Home preparation removed approximately 85 percent of the DDT residue, 96 percent of the malathion and 69 percent of the carbaryl residue. Raw, unwashed fruit stored at 55° F. exhibited no significant decrease in DDT or carbaryl. Malathion residues, however, decreased by approximately 30 percent during a 7-day storage period.

The effect of commercial and home preparation on DDT residues in potatoes grown on soil treated by from 19.1 to 23.2 lbs./acre DDT was investigated by Lamb *et al.*, (*16*). Commercial washing operations removed approximately 20 percent of the total DDT residue from potatoes whereas lye peeling plus washing removed about 94 percent. Following washing, commercial processing further reduced the residue to insignificant levels. Peeling removed more than 91 percent of the residue during home preparative procedures. No significant loss of DDT residue was exhibited by potatoes stored at 45° F. for a period of 6 weeks.

Gunther *et al.*, (*17*) found that the half-life values for Guthion under dry field conditions was 30 to 38 days for lemons and 340 to 400 days for oranges. Rainfall or washing markedly decreased the Guthion residues. Guthion is largely nonpenetrating, therefore, and relatively persistent on these fruits, but may be removed easily.

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Soil Contamination

The fall-out of pesticides following sprayed applications in the direct treatment of soil has led to a build-up in the soil of various amounts of pesticides. Soil residues may, therefore, be a cause for concern since they may reach man by a number of routes: uptake from soil by consumable crops; leaching into water supplies; volatilization into the air: and by direct contact with the soil. The magnitude of the problem is directly related to the amount supplied to the soil and the rates of pesticide degradation in the soil. The problem can be minimized by reducing the amounts of pesticides reaching the soil by more effective application procedures, by using pesticides that have a low persistence so that once they reach the soil they remain only for short periods or by the use of pesticides of relatively low toxicity.

In order to reduce the hazards of soil contamination, it is necessary to understand and utilize the mechanisms which affect pesticides persistence in soil so that for any given application the correct parameters may be measured and evaluated. The factors which must be considered include: (1) Pesticide type and formulation; (2) pesticide adsorption; (3) soil type; (4) soil moisture and temperature; (5) uptake by plants; (6) leaching of pesticides from soil by water; and (7) wind erosion.

Pesticide type and formulation.—The persistence of a pesticide is related to all of the factors enumerated above as there are many biological, chemical and physical mechanisms of pesticide removal from soil, each acting differently depending on the chemical structure of the pesticide. For example, chlorinated hydrocarbon compounds are less amenable to bacterial breakdown than are the organophosphates.

The formulation and application method used may influence a pesticide's longevity in soil. Lichtenstein (*1*), for example, found that aldrin residues on the soil surface decreased more rapidly than residues beneath the surface, no doubt because of a greater exposure of the chemical and physical effects of weathering. It was also found that granular applications of Guthion were more persistent than emulsions.

Pesticide adsorption and soil type.—The phenomenon of pesticide adsorption is intimately related to the particular pesticide in question and the type of soil in which it is found. The nature of the colloidal soil particles, whether they are high in organic content or are of clay or sandy composition, affects adsorption. The solubility of the pesticide and the pH of the soil also affect soil adsorption. Some pesticides are absorbed more rapidly under moist conditions and some under dry. Most pesticides are adsorbed to a greater extent at cool temperatures than under high temperature conditions but not all. In short, there are few general statements about adsorption that can be made concerning all pesticides. Each compound reacts to the influencing factors in an individual manner.

Soil moisture and temperature.—In addition to affecting adsorption rates of pesticides on soil particles, the moisture and temperature of the soil may also affect microbial activity. High moisture content enhances the degradative process of hydrolysis by microorganisms. Temperature also affects the biological breakdown of pesticides, the solubility of the pesticides, and the amount of volatilization which occurs.

Uptake by plants.—Some pesticides are translocated from the soil into plants or crops. Lichenstein (*2*) found that potatoes, radishes, and carrots grown on a loam soil treated with aldrin at 1 lb. per acre contained 0, 0.03, and 0.05 p.p.m. respectively. Heptachlor-treated soils yielded greater crop residues than did those treated with aldrin. Thus, this mechanism constitutes a potential exposure hazard for man since edible crops will take up pesticides from the soil.

Leaching of pesticides from soil by water.—Pesticides held in the soil may be carried by water with possible subsequent contamination of water courses, water supplies, and groundwater. Run-off after either rainfall or irrigation may physically transport particles to which pesticides adhere or the water may leach the pesticide from the soil particles. Water was percolated through soils treated with 1 p.p.m. (=2 lbs. per 6-inch acre) aldrin or parathion (*3*). A parathion concentration of 0.013 p.p.m. was found in the water percolated through the soil. This concentration decreased with time until water percolated through the soil 17 days after treatment contained only 0.001 p.p.m. No measurable amounts of aldrin were detected in water percolated through aldrin-treated soil during the same time period. The contamination of water by pesticide soil residues was in general a function of the water solubility of the particular insecticide.

Nicholson, *et al.* (*4*) studied a pond in a 40-acre watershed which contained various crops. Parathion was applied to the crops at the rate of 4.4 lbs. per acre. During the months following treatment as much as 1.9 p.p.m. parathion was found in the bond bottom mud and 0.01–1.22 p.p.b. in the water. The bottom sediment and water concentrations varied according to the application time and the amount of soil erosion.

Tarzwell and Henderson (*5*) found that the dieldrin concentration of runoff water from a grassy area treated with 2.66 lbs. per acre decreased with time. The dieldrin concentration of water following a rainfall which occurred one day after treatment was found to be 0.13 p.p.m. The concentration of dieldrin in runoff following a third rain which occurred 9 days after the treatment was 0.025 p.p.m. No dieldrin was detected in the runoff following a rainfall later in the 9th day after treatment.

Nowhere is the argument against using the permanent types of pesticides (e.g. mercury, arsenic and lead) better illustrated than in orchard areas of the United States where arsenic-containing pesticides have been in use for decades. In many such areas the arsenic has accumulated in the soil to the point where the soil is toxic, shortening the life of trees and making difficult the profitable use of orchard lands for the forage crops that normally follow orchards in rotation (*6*).

Wind erosion.—Another possible hazard to the general public from soils contaminated with pesticides is that of wind erosion. With appropriate conditions of soil, moisture, humidity, and wind, pesticide residues from the soil may find their way into the air and be transmitted for great distances from the original source of application. (See section C, The Air Route.)

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HOUSEHOLD USE

The use of pesticides for domestic purposes has become widespread. Accompanying this increased application is an increased danger of misuse, accidental poisoning and increased contamination of the home environment. Homeowners are seldow acquainted with the scientific rationale of safe application and frequently fail to read and understand the instructions contained in the label. Thus, problems of overuse and misapplication have reached the point where contamination by household pesticides may constitute a significant proportion of the total population exposure.

Of special concern is the development of a large number of pesticide dispensing devices intended to simplify the reduction of pests in the household. Shelf paper impregnated with pesticides and evaporators intended to produce an insecticide aerosol for insect control are examples of potentially hazardous installations in closed environments. Pesticides have been incorporated in paints, furnace filters, swimming pool chemicals for algae control, and in various types of automatic dispensers, all of which may provide problems of excessive pesticides in the living environment of the household. Garden hose atomizers have been developed for home use with a variety of pesticides. These devices have the potential of causing back-siphonage in which situations highly toxic pesticide materials can be introduced into the home drinking water system.

Little information is available concerning the total amounts of household pesticides used in the United States. A study of Salt Lake County, Utah, from July, 1967, to July 1968, is illustrative of the amounts of household chemicals in use and indicates the proportion of the total amount of pesticides applied that may be attributed to domestic use (*1*).

In Salt Lake County, with an area of 764 sq. miles and a population of about 440,000, a total of 200,811 lbs of pesticide were applied during the 1-year study period. The amounts used in the various types of application are as follows:

Use	*Pounds*
Domestic	102,490
Agricultural	
Farm	36,511
Commercial Applicators	18,722
Fruit	14,540
Government Agencies	12,871
Mosquito Abatement	12,871
Live Stock	2,860
Total	200,865

In this county, therefore, domestic pesticide use accounted for approximately one-half the pesticides. These amounts would vary depending on the degree of agricultural activity in a given area but illustrate the large amounts of pesticides that are being used domestically in some areas.

Of the 102,490 lbs applied domestically, the amounts of the important pesticides used were as follows:

Pesticides	*Pounds*
Arsenic	81, 783
Chlordane	5, 876
2,4,5-T and 2,4-D	5, 790
DDVP	2, 890
Malathion	1, 380
DDT	1, 108
Other	3, 663

The inorganic pesticide arsenic, therefore, accounted for approximately 80 percent of the domestic pesticides used.

A different usage pattern was found in Arizona by the Arizona Community Pesticide Studies Project which contrasted the domestic usage of pesticides with agricultural usage for the year 1968 (2). The findings were based on 475,362 households in the state and a crop acreage of 1,204,000 acres and are presented below.

Pesticide group	House and garden use—1968	Agricultural use—1968
	lbs. tech. material	*lbs. tech, material*
Chlorinated hydrocarbons	28, 600	4, 202, 000
Herbicides	12, 690	870, 000
Organophosphates	11, 590	2, 839, 200
Miscellaneous insecticides	5, 600	263, 600
Fungicides	2, 600	126, 000
Carbamates	1, 900	153, 400
Defoliants and dessicants		1, 084, 100
Total	62, 980	9, 538, 300

Thus, in contrast to the findings in Salt Lake County where a high arsenic domestic usage was found, the group of chlorinated hydrocarbon pesticides accounted for approximately 45.5 percent of the pesticides used domestically. Also, whereas the domestic usage of pesticides in Salt Lake County accounted for approximately 50 percent of the total, domestic usage in the state of Arizona accounted for only about 0.6 percent of the total.

All people, of course, do not use pesticdes in their homes and gardens in equal amounts. Finklea *et al.* (*3*), for example, surveyed Charleston, South Carolina, and found that whereas 83 percent of the white families sampled used pesticides in their home, 97 percent of the nonwhite families employed household chemicals for pest control. It was observed that the homes of nonwhite families were more frequently infested with roaches, mosquitoes and flies. Nonwhites therefore applied pesticides inside their homes more intensely than did whites. Yard and garden applications, however, were found principally among whites. Thus, the differences in application were probably attributable to differences in economic status and housing quality.

Both white and nonwhite families commonly ignored safety precautions in the use of household chemicals. Locked storage was not employed by 88 percent of all families; 66 percent stored the pesticides within easy reach of small children; 54 percent stored the chemicals near food or medicine; and 66 percent never wore protective gloves during use or washed their hands after the application.

This study indicates that lower socioeconomic groups may use pesticides in greater amounts than higher socioeconomic levels but that both groups tend to disregard safe practices in the application and the storage of these poisons.

Davies and Carter (*4*) observed differences in DDE blood levels in children living in different areas of Dade County, Florida. Some of the children in the poorer sections exhibited DDE blood levels as high as those found in adults. The authors hypothesized that the pesticide blood levels could be correlated with the dust levels in the homes, as the poorer homes tended to have higher dust levels than other homes. Poorer homes had many roaches and therefore a greater dependence on insecticides prevailed in these homes. Also, since DDT is one of the cheaper pesticides, the poorer families favored its use. The younger children, therefore, would be most likely to be exposed to the dust as they crawled upon the floor. To test this hypothesis, kittens were placed in a clean home in good repair and in one of the poorer homes. The kitten in the clean home exhibited no increase of DDT and DDE blood levels during the study period of four months. The kitten in the poorer home, however, exhibited a rise of DDT from 12 p.p.b. to 120 p.p.b. and in DDE from 10 to 20 p.p.b. during this same period. The average blood DDE in the four children in this house was 39 p.p.b. Both homes were given the same cat food. Therefore, even though the number of kittens in the experiment was not large, a demonstrable difference in blood DDT and DDE levels was observed, indicating that general household conditions may have an effect on the possible exposure to children when all other factors are equal.

Several studies have attempted to correlate environmental pesticide levels attributable to domestic usage with pesticide blood levels or respiratory performance. Weiner and Worth (*5*) studied two groups with similar income, educational level, occupations, residences, and ethnic background to determine the relationship between insecticide use and respiratory impairment. The heavy-use group applied pesticides once a week or more often and the light-use group used pesticides less than once a week. Tests of forced expiratory volume and forced vital capacity revealed that people in the consistently light pesticide use group performed significantly better than those in households using insecticides heavily. There was no definite correlation between respiratory impairment and a particular pesticide nor could any other environmental variable be attributed to the differences in respiratory function. More asthma and chronic sinusitis were observed in the heavy-use group and perennial nasal allergy was twice as common in this group.

A comparison of the exposure to lindane from home vaporizers with that of occupational exposure levels is provided by a study in California (*6*). Several groups of people, each having various exposures to lindane were studied. Lindane blood levels and air concentrations to which they were exposed were sampled. The results are summarized below:

Sample population	Lindane in whole blood—p.p.b.		Lindane air concentration—mg×10^{-3}/m^3
	Mean	Range	
Control—No exposure	0. 46	0. 3–0. 9	--------------
Nonproduction workers in lindane plants	0. 93	0. 3–2. 5	9. 0–49
Production workers in plant 1 with little or no skin contact	4. 6	1. 9–8. 3	31–1800
Production workers in plant 2 with little or no skin contact	4. 1	1. 0–8. 9	11–1170
Production workers in plant 2 with ample skin contact	30. 6	6. 0–93. 0	11–1170
Lindane exposure from home vaporizers only	2. 2	0. 9–5. 2	1. 0–110

The exposure to lindane home vaporizers produced greater blood concentrations than nonproduction workers experienced in lindane plants and in some cases, as evidenced by the overlapping ranges, produced blood levels higher than in production workers in lindane plants. Lindane air concentrations attributable to lindane home vaporizers were at times as high as those concentrations in the lindane manufacturing plants.

In summary, the data presented indicate that the use of household pesticides, while high for all groups, is greater in lower socio-economic levels than in high. This fact, together with the generally less clean conditions found in poorer homes, indicates that people of lower economic levels may receive a higher exposure to household pesticides than other segments of the population. All groups tend to disregard the labeled instructions for safe application and storage. Respiratory impairment and high blood pesticide levels have been correlated with heavy pesticide use.

An additional potential household exposure is provided by contact with wearing apparel that has been treated with pesticides for mothproofing. A survey of dry cleaning establishments in three counties of Mississippi was conducted by the Mississippi Community Studies Pesticide Project in an attempt to detetrmine the number of firms which employed such practices and the pesticides involved (*7*). Forty-one dry cleaning firms were questioned about their mothproofing procedures during the year 1968. Of the dry cleaners studied, 16 used no mothproofing agents while 25 did use such products. When mothproofing agents were employed, it was generally the practice to mix a quantity of the agent with the regular dry cleaning fluid. Thus, in these firms, every article of clothing which was dry cleaned was mothproofed as well, regardless of whether the customer had requested mothproofing. Mothproofing generally began in April or May and ended in July, August, or September. One dry cleaning firm used mothproofing year round.

Four main mothproofing chemicals were used: Sanex, Milo, Tripruf, and Sanitone. All four products contained DDT, although Sanitone, which was 100 percent DDT, was the only product in which the DDT concentration was identified. The maximum volume of mothproofing chemical used during 1968 by any firm was 20 gallons; the maximum weight was 75 pounds.

In most cases, only one or two employees in a given firm had direct contact with the mothproofing chemical. The estimated number of families affected, however, was always in the hundreds, and in some instances, it was in the thousands. A large number of customers was therefore exposed to DDT by the mothproofing practices of these dry cleaning firms.

Commercial pest control.—Throughout the nation there are large numbers of commercial pest control operators who work extensively in private dwellings and institutions in the control of household pests. In 1963 there were 5223 firms concerned with structural pest control and exterminating services and the number continues to grow. The annual gross income from these operations is estimated to be over 450 million dollars of which about 30 to 40 million dollars is spent on the purchase of pesticides (*8*).

The 10 most important pests controlled by pest control operators in 1965 were as follows:

1. German roach.
2. House mouse.
3. Norway rat.
4. Subterranean termites.
5. House ants.

6. American roach.
7. Carpenter ants.
8. Oriental roach.
9. Fleas.
10. Brown dog tick.

The German cockroach and the two commensal rodents are the three most important pests encountered and require frequent, usually monthly, control. Although fourth on the list of important pests, termite control accounts for about 35 percent of the industry's income.

The most important insecticides for household use are as follows:

1. Diazinon spray.
2. Chlordane spray.
3. DDVP Spray additive.
4. "Kepone" pellets.
5. DDT dust.
6. Pyrethrins and synergist.
7. "Baygon."
8. Sodium fluoride.
9. DDVP spray.

Diazinon is an organophosphate, is the most frequently used insecticide for indoor work and is effective against most chlorinated hydrocarbon resistant roaches. Chlordane is very effective against ants and most species of cockroaches and is used for the control of many minor pests because of its long residual action, low odor and moderate mammalian toxicity. DDVP is used to provide a quick knockdown in conjunction with more persistent chemicals such as Diazinon and chlordane. The use of Kepone pellets as baits has increased even though it is relatively slow acting. DDT dusts are used as a trailing powder to control mice and for the control of cockroaches. Pyrethrins are used as knockdown sprays and in food processing and storage. Baygon, a carbamate, is used to control chlorinated hydrocarbon resistant cockroaches. Sodium fluoride is often mixed with pyrethrum for dusting walls and concealed areas.

Anticoagulants, such as Kepone pellets referred to above, are used extensively by pest control operators for the control of rodents. Their use is primarily as a bait to maintain low population levels after the rodent numbers have been reduced. Water solutions of anticoagulants are sometimes used, as are tracking powders, but the most common method employed is that of solid baits. Although anticoagulants are slow acting, their low toxicity makes them quite attractive from the safety standpoint. These compounds are ineffective against some rodent populations, however, because of a natural tolerance or resistance and more toxic rodenticides are required in these situations.

Proper commercial pest control dictates that the chemical and target pest organism be matched effectively. The pesticide which will accomplish the greatest kill in the shortest possible time and in the safest manner is the chemical which should be used. The toxicity of the pesticide must be balanced with its persistence, however, since many pest organisms require frequent control. Nonpersistent pesticides may result in uneconomical control frequencies as, for example, in termite control. More persistent pesticides are therefore often required and their application must be properly administered to avoid long-term contamination.

Most commercial pest control operations are under the licensing jurisdiction of local units of government and in some cases under State control. The operators, in the main, have demonstrated an interest in the protection of the health of their employees as well as protection of the health of the public. The National Pest Control Association, an organization composed of and sponsored by commercial pest control operators, promotes the safe use of pesticides and helps in the production and evaluation of new chemicals and control techniques.

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Pesticide Manufacture, Occupational Exposure and Accidents

In the processes of manufacturing and formulating pesticide materials, industrial employees may be exposed to pesticide gases, fumes, and dusts, and to skin contact with these chemicals. In many of the large manufacturing plants, industrial hygiene and medical supervision are available. Among the small manufacturers throughout the country, however, little attention is given to industrial hygiene practices and medical supervision is not available.

Hazards to manufacturing employees can be reduced through a variety of methods, as for example, the installation of appropriate ventilation equipment. Hartwell and Hayes (*1*) observed workers in two organophosphate formulating plants, one with adequate and the other with inadequate facilities for respiratory protection against inhalation of airborne contaminants. In both plants, the workers who loaded and packaged the formulation mixture, the most hazardous job in the manufacture of pesticides, were studied. Cholinesterase activity depressions as a result of pesticide poisoning occurred 41 times in 26 subjects in the plant with inadequate protection during the first year of the study. When the plant installed a system which distributed uncontaminated compressed air to facemasks worn by each worker, the incidence of pesticide poisoning decreased drastically. The other plant studied had such a compressed air system but poisonings occurred due to a faulty design in the air system which allowed a back syphonage of pesticide-laden air to occur. When this situation was corrected, the incidence of pesticide poisoning decreased.

Protective clothing may also be employed to protect the workers. The training and instruction of the workers regarding the hazards involved in the manufacture of the pesticide, the techniques used for self-protection, the reasons for this protection and the procedures to be followed in case of accidents are perhaps the most important preventive actions.

It is well known that workers in plants producing persistent pesticides, such as DDT, accumulate much higher levels of the pesticide in their adipose tissue than the general population. However, Laws *et al.* (*2*) found that even though 35 workers with exposures of more than 11 years at a DDT manufacturing plant had accumulated 38 to 647 p.p.m. DDT as compared to 8 p.p.m. in the general population, no ill effects were evident. The long-range effects of such high exposures and accumulation rates are less certain.

Agricultural applications.—In California during 1966, 1,347 accidents were attributed to pesticides and other agricultural chemicals (*3*). Accidents in the agricultural use of these chemicals numbered 820 while all other occupations accounted for 527 accidents. The number of accidents involving these chemicals in terms of the industry where the accident occurred and the occupation of the injured persons is reported below.

Agricultural Chemical	Accident total	By industry			By occupation	
		Agricultural	Manufacturing	Other [1]	Farm laborers	Other [2]
Total	1, 347	820	204	323	704	623
Organophosphates	253	183	46	24	152	101
Parathion	102	88	12	2	79	23
Other	151	95	34	22	73	78
Chlorinated Hydrocarbons	94	49	18	27	42	52
DDT, chlordane, lindane, kolthane	40	19	7	14	17	23
Methyl Bromide	30	17	6	7	17	13
Other	24	13	5	6	8	16
Lead and/or arsenic compounds	10	7	1	2	7	3
Herbicides	145	90	12	43	80	65
Fertilizers	133	73	37	23	48	85
Other	[3] 712	418	90	204	375	337

[1] Includes: Construction; transportation; trade; structural pest control; government; and unspecified.

[2] Includes: Professional; clerical and sales workers; truck drivers; gardeners; and unspecified.

[3] Includes: Fungicides; carbamates; sulfur; and 438 unspecified.

Of the accidents caused by known compounds, the organophosphates were involved more than any other group. Of these, parathion accounted for approximately 40 percent. Herbicides and fertilizers, with approximately the same number of accidents, were involved in the next highest number of accidents where the pesticide or chemical was specified. Chlorinated hydrocarbons were involved in the fourth largest number of accidents. Within this group, DDT, chlordane, lindane, and Kelthane accounted for approximately 43 percent of the cases and methyl bromide, 32 percent.

There were approximately four times as many accidents involving agricultural uses as there were industrial accidents. More agricultural accidents than industrial accidents occurred with every pesticide group listed.

Farm laborers were involved in more accidents than all other workers combined, accounting for approximately 52 percent of all reported accidents. The accident rate for each pesticide specified was higher for the farm laborer category than for any other working situation.

Exposure to pesticides from agricultural applications may be experienced by individuals who apply the pesticide or by agriculture workers who work in the fields where the pesticide has been applied. Wolfe *et al.* (*4*) studied the health hazards associated with the agricultural application of endrin and dieldrin, two of the more toxic chlorinated hydrocarbons. The dermal exposure of workers wearing normal clothing while spraying a 1-percent formulation of endrin dust on potato fields was calculated to be 18.7 mg./hr. The respiratory exposure was calculated to be approximately 1.8 percent of a toxic dose per hour of exposure. While spraying endrin on orchard cover crops and dieldrin on pear orchards, the calculated exposure was 0.2 percent and 0.3 percent, respectively, of a toxic dose per hour. Even though the exposure levels were far below toxic levels, the fact that chlorinated hydrocarbons are stored in the body fat must be taken into account when evaluating the hazards of repeated exposures.

It has been estimated that an average orchard sprayman who wore no hat or protective clothing on his arms would be exposed to approximately 7.7 percent of the toxic dose of parathion per hour (*5*). After about 13 hours of spraying, therefore, the individual could theoretically become poisoned. Experience has shown that this is not necessarily true, however, and this may be attributed to the slow and/or incomplete absorption of the pesticide which impinges on the skin. Therefore, toxicity data must be tempered with knowledge of the mechanisms of pesticide entrance into the body.

Wolfe *et al.* (*6*) determined values for dermal, respiratory, and total exposure in terms of the fraction of toxic dose for 31 different work activities associated with the pesticide application of 10 different pesticides. Wind was the most important environmental condition studied, as the amount and direction of wind directly affected exposure levels. For each pesticide, there appeared to be a significant variation in the hazard depending on the type of activity in which the worker was engaged. For example, the hazard from the indoor house spraying of DDT was about four times as great as flagging for airplane dusting of fruit orchards, about seven times as hazardous as outdoor house spraying, and over 30 times as hazardous as operating an air blast spray machine in a fruit orchard. The loader received about three times as much exposure as the pilot and about 4½ as much as the flagman.

The method and rate of application also affected the potential exposure. For example, the potential exposure while operating an air blast machine spraying tree orchards with parathion was about 12 times as great as the exposure when spraying the same compound on row crops with a boom-type sprayer. The air blast machine sprays the pesticide up into the air where it is more subject to drift whereas the boom-type sprayer directs the spray downward. Increases in the application rate would also be expected to increase the possible exposure. In the same way, the amount of time the workman worked at his particular job would also increase the pesticide exposure.

The potential dermal exposure to each pesticide was much greater in every work situation studied than the potential respiratory exposure. The respiratory exposure for the various work situations studied ranged from 0.02 to 5.8 percent of the total exposure. The use of low-volume concentrate spraying of parathion in fruit orchards resulted in a respiratory exposure about three times as great as that observed for similar parathion applications using conventional high-volume spray. This is undoubtedly due to the fact that the low-volume concentrate spraying results in particles of significantly smaller size than conventional sprays.

Only three of the compounds studied, endrin, parathion, and TEPP, were involved in operations in which the mean value for the percentage of toxic dose potentially absorbed per hour exceeded 1 percent. The highest potential exposure was observed for workers who loaded airplanes with 1 percent TEPP dust. They received 44.2 percent of the toxic dose per hour of work. Although the fraction of toxic dose received during application of some of the less toxic chlorinated hydrocarbon pesticides was comparatively low, these compounds are, as noted previously, stored in the body fat following absorption. No manifestations of such pesticide exposure have been observed, however. In cases where poisoning did occur, it was possible to show an obvious disregard of one or more of the safety regulations. Thus, this study affirms that pesticides can be used safely provided recommended precautions are followed.

Quinby and Lemmon (7) studied the potential exposure of workers engaged in picking, thinning, cultivating, and irrigating various crops to which parathion had been applied. Eleven cases of poisoning involving more than 70 persons occurred from contact with the parathion residues. Since the air concentration of parathion over the crops was extremely low, it appeared that most of the affected workers were exposed primarily by the dermal rather than the respiratory route. Also, it was observed that many of the workers removed their protective clothing and wore contaminated clothing for long periods of time. Thus, these poisonings were a direct result of working with and handling contaminated crops.

In all cases where individuals work with pesticides, it is essential that they be made aware of the problems peculiar to the particular chemical exposure and that they become familiar with methods of protection.

Transportation.—In the consideration of problems associated with contamination of the environment, the possible contamination through the transportation of pesticides from the point of manufacture to the point of final application must be considered. The equipment designed for transportaion of large volumes of pesticide, which in case of accident could cause hazard to numbers of individuals, require a design such that accidents would be prevented insofar as possible. Tank cars, trucks, and pesticide containers need to be designed in such a manner that mishandling, which frequently occurs in shipment, will not cause accidents to the individuals handling the material or to the general public. It is essential that equipment used in handling and disseminating pesticides be adequately and properly maintained, so that spills and the escape of pesticides is avoided.

There is also a need to prevent food contamination with pesticides, particularly in shipment. A number of cases have been reported in which pesticide materials shipped with foodstuffs contaminated large volumes of food, thereby causing subsequent illness and death.

To minimize such occurrences, the simultaneous shipment of pesticides and foodstuffs should be avoided. In fact, vehicles used for bulk pesticide shipment should be single-use vehicles; i.e., their use should be confined solely to the transport of pesticide chemicals.

Contamination of water supplies by accidental spills and the contamination of grain used subsequently to make flour has also been experienced. In all cases, consideration must be given to the instruction of the individual handling the material concerning the hazardous nature of the chemical involved.

Accidents.—A large number of accidents from pesticides occur each year. For example, in 1961, 8.5 percent of all cases of accidents and poisoning of children younger than 5 years of age in the United States was attributed to pesticides (*8*). Of the 32,034 reported cases, 16,119 were caused by medicines, 1,726 by petroleum products, and 2,709 by pesticides.

In South Carolina, between 1957 and 1966, 38 deaths were attributed to pesticide usage (*9*). Most poisonings occurred in areas of high pesticide application and 60 percent of the fatalities occurred in children under 5 years of age. Parathion accounted for 34.2 percent of the poisonings and arsenic 31.6 percent.

In Dade County, Fla., studies of the pesticide mortality records for the years 1956–67 revealed that children under 5 years of age accounted for 23 percent of the fatal poisonings (*10*). These deaths were primarily due to accidental ingestion of the pesticides in and around the home. In addition to the children, young to middle age adult males who were occupationally poisoned and middle age to older adults who suicidally ingested pesticides accounted for the majority of the fatal and nonfatal pesticide poisonings. Organophosphate pesticides accounted for 53 percent of the fatal poisonings.

McLeod and Herban (*11*) studied the pesticide mortalities which occurred in Louisiana between 1958 and 1967. Pesticides were the cause of 102 deaths during this period. The 10-year cumulative death rate from pesticides per 100,000 population are as follows:

White:	
Accidents	0. 48
Suicide	1. 94
Nonwhite:	
Accidents	2. 21
Suicides	0. 62

Pesticide ingestion accounted for 75 out of the 102 cases. Most of the poisonings in infants and children were accidental whereas poisonings in adults were primarily suicides. Only three deaths were occupational and these were all agricultural. Arsenic accounted for 37 deaths; unknown pesticides 21; inorganic phosphorus 12; fluoride 10; and organophosphates 9.

McLeod (*12*) also studied the 107 cases of pesticide poisoning in patients admitted to Charity Hospital in New Orleans from July 1965, through June 1967. Children less than 5 years of age accounted for 77.6 percent of these cases. In comparison with the total admissions to the hospital, a greater proportion of the pesticide poisoning patients were Negro. Most of the adult poisonings were the result of suicidal attempts whereas the poisoning of children occurred through accidental ingestion. Pesticide mixtures accounted for 26.1 percent of the poisonings; Fumarin and Warfarin in 20.6 percent; organophosphates 15.9 percent; and chlorinated hydrocarbons 4.6 percent.

The above studies indicate that children who accidentally ingest pesticides in the home account for a large proportion of the accidental poisonings attributable to pesticides. Efforts aimed at the reduction of accidental poisonings, therefore, must be directed toward the minimizing of children-pesticide contact in the home environment. No single pesticide or pesticide formulation has been incriminated as being a major cause of accidental poisoning. The availability or ease of access to the pesticide would seem to be more important than the type of chemical involved.

Accidental exposures to pesticides occur through inhalation, direct consumption of contaminated food, and through skin absorption. Coble *et al.* (*13*) reports the accidental poisoning of several people by flour contaminated with endrin. Although no human fatalities resulted, the bread which caused the poisoning killed a rat within 12 hours after feeding and caused dogs to convulse. Extracts from the flour sacks killed a dog 2 hours following ingestion. How the flour became contaminated was not known.

The packaging of pesticide products is an important consideration in the reduction of accidents, whether in the manufacturing plant, during shipment, in stores or in homes. Defective packages or packages which might rupture during rough handling must be avoided. The use of package materials which will not weaken during long periods of storage due to degradative processes such as corrosion should be made mandatory.

Another problem is that of the disposal of used pesticide containers. Large containers may contain appreciable amounts of pesticides after use. For example, Wolfe *et al* (*14*) found that 22 empty 5-gallon metal drums which had contained 45.6 percent parathion emulsifiable concentrate contained an average of 2.73 gm. of technical parathion in suspension. Tests indicated that rinsing the containers twice with water removed almost 98 percent of the removable parathion. The disposal of the rinse water presents additional problems, the solution to which may be the shipment of unrinsed bulk containers back to the manufacturer who, presumably, would have the facilities necessary to clean the containers, dispose of the rinse water and dispose of or reuse the containers, and at the same time insure that the containers would not be reused for packaging foods or the like.

The incorrect disposal of containers used for household pesticides constitutes a hazard to man and his environment. It is important, for example, that glass or metal containers be disposed of soon after their contents have been emptied in order that they may not be used for drinking water jugs or other household purposes or remain available for possible contact with children. The present day disposal methods, namely incineration (as presently practiced, time-temperature aspects are insufficient for pyrolysis) or burial in the ground, are unsatisfactory in that even though they minimize the immediate dangers of exposure, they contribute to the buildup of environmental contamination. A solution offered for the disposal of bulk containers may not be practical with regard to the many smaller bags, bottles, boxes, cans, etc., sold to the public each year. Obviously, a more satisfactory solution to this problem must be found. In this connection, information as to the proper disposal of old, unsold stock or of discontinued pesticides should be made available to all pesticide users.

The accidental incorporation of massive levels of granular or powder insecticides in animal feed in place of the intended mineral is a primary source of pesticide problems in livestock. Animals that die from such exposures are disposed of in rendering plants and end up in meat scraps and tankage fed to animals, thus contributing to pesticide residues in foods for humans.

In order to prevent accidents of the types listed above, one of the important aspects is that of labeling, coloring, or otherwise clearly marking pesticides in such a manner that will bring to the attention of users the hazards which exist. It is important that the user of pesticides be thoroughly and forcibly warned of the hazardous nature of the material and the reasons for the precautions which are recommended. Problems of storage need to be given adequate attention, particularly in relation to storage of household pesticides so as to prevent their availability to children.

The possibility of sales control through appropriate registration of the more hazardous pesticides should be given attention.

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Alternative Pest Control Measures

Alternatives to persistent pesticides may be of two main types. First, nonpersistent pesticides may be substituted for persistent materials. Secondly, pest control procedures which do not utilize toxic chemicals may be employed. Such alternative methods are usually of a biological or physical nature but their use has been limited up to this time, although much research has gone into the development of these techniques. An increased emphasis is being placed on nonpesticide control methods, with more than 50 percent of the Agricultural Research Service entomological research budget in 1967 devoted to these alternative techniques. Since more than 90 percent of the total amount of pesticides used are employed for the control of fewer than 100 species of pest organisms, the reduction of these specific pest populations by nonchemical methods would significantly reduce the pesticide burden.

In some situations, the establishment of a goal for pesticide treatment of less than a 100 percent kill would not only reduce the amount of pesticide needed but also would allow the maintenance of beneficial predators in the community enabling them to continue exerting a natural population control of the pest organisms.

Development of nonpersistent pesticides.—The development of nonpersistant pesticides has had high priority for the last several years. The results of this intense effort are already becoming evident as the trends in pesticide application are to the use of the less persistent pesticides. For example, the organophosphate compounds are undergoing increased production and use whereas the persistent chlorinated hydrocarbon compounds are, in general, declining in use. Although many of the less persistent pesticides are more toxic than the persistent chemicals, and therefore can exert a greater potential toxic effect on pesticide handlers and on nontarget organisms, the fact that they remain viable in the environment for shorter periods of time reduces the possibility of their entrance into ecological systems with possible harmful consequences.

Resistant crops.—One of the best methods for protecting crops against insect injury is the development and use of resistant plant varieties. The expense of applying sprays and dust, the hazard of chemical residues, and the danger of developing resistance to pesticides are all avoided. The development of resistant crops, therefore is a continuous process of discovery, breeding, and selection of resistant strains. As the diseases are constantly changing, the resistance of crops to plant diseases may also vary. Rainfall, temperature, fertility, planting dates, and soil condition all influence the degree of resistance shown to a crop disease.

Resistant plants that have been developed frequently show an improvement in yield and quality as a result of more normal growth. Various strains of wheat, oats, and barley and other grains have been developed which are resistant to leaf blights and rusts. Various strains of nongrain plants such as alfalfa, cotton, tobacco, beans, potatoes, and tomatoes have been developed which are resistant to specific diseases caused by various fungi, bacteria, viruses, and nematodes. The goal is the development of strains of each type of crop which are resistant to all major types of diseases.

Plants which are resistant to attack by insects, as for example the Hessian fly, have been developed. Corn varieties are available that reduce cornborer losses by 70 percent. Wheat and alfalfa strains which are resistant to the particular insects which attack these crops are being developed and cotton strains resistant to attack by the boll weevil have been found. The development of resistant varieties appears to be the most successful of all nonchemical control methods.

Insect parasites and predators.—The introduction of beneficial parasites and predators not native to a particular location and their artificial growth and distribution is one method by which pests may be controlled. In addition to the potential lower pest control costs and avoidance of chemical residues, the widespread substitution of parasites or predators for chemical applications would aid in preventing the danger of developing insect resistance and would also reduce the possible pesticide injury to the host plant.

There are now at least 95 species of imported parasites and predators established in the continental United States. Many of these were brought from Europe to control the gypsy moth, the brown-tail moth, the European cornborer, and the alfalfa weevil. A large majority of the introduced species are parasites. There are at least 32 species of parasites used in California against scale insects and mealy bugs. Tachinid flies are used principally against forest and shade tree insects. The development of techniques which allow for an economical production of great numbers of parasites has been a large factor in the success of these efforts. This is necessary so that effective numbers can be released in areas infested by pest insects.

In spite of some success, there have been only 106 insects anywhere in the world including 46 in the United States, that have been partially, substantially or completely controlled by parasites or predators introduced into an area by man (*1*). For reasons of easier geographical and ecological control, the greatest successes in this type of treatment have occurred on islands. This is also true of other means of biological control and it is a fact that large volumes of pesticides still must be used to control the organisms to which biological control methods have been applied despite some success.

Insect pathogens.—Since insects are subject to infection by disease-causing organisms, namely, bacteria, viruses, fungi, protozoa, and nematodes, the use of these organisms offers potential control of pest insects. The organisms which attack insects are very seldom the same organisms which attack higher plants and animals, which make them relatively specific by nature. Various attempts have been made to develop control methods by utilizing disease-producing organisms. Studies have been made on chinch bugs, grasshoppers, the browntail moth, and the citrus white fly, using fungi as a disease-producing agent. The propagation and distribution of fungi, however, is largely dependent on weather conditions and the results of field studies have been difficult to evaluate. Another problem with the use of insect fungus diseases is that atmospheric moisture and temperature greatly affect their dissemination. Warm humid conditions are usually required for the use of fungi in the control of insects. Bacteria, viruses, and protozoa, on the other hand, are not so dependent on high relative humitity for growth and dissemination as are the fungi. These organisms may be ingested with food or carried by the insect's predators and parasites, by wind and rain and irrigation water. Weather conditions are important, however, in developing the population densities required for the control of pest organisms.

Bacteria have been utilized for the control of the Japanese beetle. Large numbers of beetle grubs are first infected with the bacterium *Bacillus sp.* The grubs are then refrigerated until ready for use. At that time, the grubs are ground up, a dust base is added, and the spore powders are distributed through areas infested with Japanese beetles. The bacteria spores are resistant to dryness, moisture, cold and heat, and may live in the soil for years infecting grubs feeding on grass roots.

The control of the alfalfa caterpillar has been accomplished by infection with the virus *Borrelina campeoles.* Infection of these caterpillars by this virus can reduce caterpillar populations below economically important levels in from 5 to 7 days. Supplies of the virus may be augmented by the continued collection of infected larvae in the field and their processing to conditions amenable to dissemination.

Micro-organisms used for insect control should not pose a threat to higher animals or plants. The main difficulties of this type of control involve the manufacture and dissemination of large amounts of the microbial material. Also, most of these biological agents are specific by nature and affect only one pest, allowing other pest insects to live and flourish once their competition has been reduced. Pathogens also generally do not produce the quick kills which farmers prefer. Thus, despite some promise, the pathogenic control of pest organisms must be improved substantially before it can become an effective control technique.

Cultural or environmental control.—The so-called cultural or environmental techniques for pest control are essentially modifications of the ecological systems within which the insects operate. One such approach is the use of induced sexual sterility in which the basic behavioral patterns of the insect provide the mechanism of insect control. In this technique, insects which are sexually sterilized are released to mate with normal insects thus reducing the population's reproductive potential. If the number of sterile matings is sufficiently greater than the number of normal matings, the population will decrease with each generation instead of increasing. When, by continued release, the number of sterile insects is maintained at a constant level, while the number of normal insects declines, the ratio of sterile matings to normal matings will increase rapidly in successive generations.

In many eradication programs, such as the screwworm and melon fly programs, sterility was induced by irradiation. Since this technique requires the rearing of insects in the laboratory in large numbers, it is not applicable to species which are not suited to such laboratory rearing. In these cases, chemical sterility of a large proportion of the existing natural population would provide the answer. Chemosterilants are effective in the control of species in which the males and females that are produced over a sizable area mix thoroughly before mating takes place. If both sexes remain near the site at which they molt to adults until after mating has taken place, chemosterilants would not be as effective as toxic chemicals. All of the chemosterilants which are presently in use are presumably mutagenic agents. For this reason, contact between these chemicals and man or beneficial animals should be avoided. Species that are attracted to baits and other attractants are particularly susceptible to such methods. As of 1966 chemosterilants were not recommended for the control of any species and no large scale experiments in their use had been conducted. Research was under way to develop safe chemosterilants and to devise safe and effective methods of application.

Regardless of whether the sterility is induced by chemosterilants or by irradiation, there are certain characteristics necessary for a species to be controlled by sexual sterilization. The first necessity, particularly in reference to air-radiation produced sterility, is that the species be able to be raised economically in large numbers. Species that produce a large number of eggs per female and have a short life cycle are most readily amenable to this type of rearing. However, it is also essential that the sterilized insects do not in themselves constitute a nuisance or a source of injury. Such insects as houseflies and cockroaches, or insects which serve as disease vectors, are not species that can be acceptably controlled by sexual sterility. Species which are widely distributed or have economic significance also would not be practically controlled by sexual sterility. Sexual sterility will achieve maximum success only with species in which the males and females mix over a considerable area before mating occurs. If the insects are restricted in their movement it may be difficult to obtain adequate distribution and placement of sterile insects in required numbers to compete successfully with the normal insect in the various parts of the total area. And finally, it is necessary to be able to sterilize the species without too serious effects on its vigor, longevity, behavior, or mating competitiveness.

Another method of effective sterilization of insects is to inject into the adult insects synthetic juvenile hormones. Juvenile hormones are produced by the insect's body during metamorphosis to retard the development of the adult characteristics. During the final stage of metamorphosis the juvenile hormone is not produced, allowing adult characteristics to develop. When large amounts of synthetic juvenile hormone are introduced into adult insects, the insects are effectively sterilized by the degeneration of the adult characteristics. Williams and Robbins (*2*) report on research by Röller which determined that 20 mg. of synthetic juvenile hormone was fully effective in controlling codling moth infestations in individual apple trees. In other experiments it was found that when the high dose of 1 mg. of synthetic juvenile hormone was injected into a body of an individual male adult of *Pyrrhocoris apterus*, normal females received sufficient juvenile hormone to sterilize them when mated to the treated male. The contaminated females were then in turn able to infect normal males during later matings, and in many cases, these infected males passed along sufficient hormone to sterilize yet a further group of normal females. Thus, if this technique could become effective, the dissemination of the sterilizing chemical would be enchanced by the normal activity of the insects themselves.

However, the juvenile hormone may be considered a broad-spectrum insecticide with a potential for killing beneficial fauna, and hence, care in its application must be exercised.

Physical pest control methods.—Various types of physical pest control methods have been developed and are undergoing study in order to develop their practicality and usefulness. Nelson and Seubert (*3*) reviewed new methods of pest control which utilized forms of electromagnetic, sonic, and ultrasonic energy.

The use of radiofrequency (RF) electromagnetic energy for the control of insects has been investigated. Although it has been postulated that RF energy of some particular frequency would be effective in killing insects by virtue of some resonance phenomenon, this has never been demonstrated. It is thought the main lethal action of RF energy on insects is produced by the heating of the RF electric fields. The use of RF heating in the control of insects in a large area is impractical. The main application of the RF heating effect has been in the insect control of grain, foodstuffs, and wood. RF heating has been used to control insects in stored grain without damage to the grain itself. RF heating has been used for wood disinfestation where in some cases it might be more practical than conventional heating and chemical methods by virtue of its deeper penetration. Little of the RF energy spectrum has been studied but from the results obtained thus far this type of electromagnetic energy merits further investigation.

Infrared energy has been used for insect control by radiating grain on conveyor belts and maintaining lethal temperatures. The use of infrared energy for the control of insects within large areas has not proved practical. Attractants which utilize infrared light sources have had some success.

Visible light has been used for many years to attract insects. Most of the common light traps employ blacklight, fluorescent lamps as the attractant source. These lamps emit strongly in the ultraviolet region and attract large numbers of photopositive insects. Light traps have been used as an entomological survey device, for detection and quarantine work, for the detection of population changes, and the prediction of insect infestations. The use of light traps for the direct control of insects has been effective in small areas such as vegetable crops or garden plots and there has been some evidence which suggests that the control of insects in larger areas by this method may be possible. Light traps at a density of three traps per square mile over a circular area 12 miles in diameter reduced the tobacco hornworm population by approximately 65 percent in one season. Light traps in combination with other techniques offer promise. With certain insects, light traps containing caged virgin females exhibited a higher catch of male insects than either separately. Light traps might possibly be used in combination with chemosterilants.

Visible light may be used in other ways to control insects. For example, during critical times in the life cycle of certain insects, if the normal dark period is interrupted by a momentary flash of light, normal development is prevented. More research into the seasonal cycling and development of individual insect species must be undertaken so that techniques such as this can be used effectively.

X-rays and gamma rays, located at the high-energy end of the electromagnetic spectrum, are extremely penetrating types of radiation. The effect of their ionizing tissue atoms can be very damaging if sufficient energy absorption results from radiation exposure. One use of ionizing radiation in insect control is that of the sterilization of male insects as discussed previously. The use of irradiation for disinfecting grain has also received much attention. Various dose levels applied to grain are used to either sterilize or kill insects without harming the grain. Wheat irradiation with 20,000 to 50,000 rads from gamma sources providing energies of not greater than 2.2 Mev. has been approved by the Food and Drug Administration. Since studies of gamma irradiation of citrus fruits have shown that low doses of radiation are effective in controlling eggs and larvae of the Mexican fruit fly, approval of gamma irradiation for such fruits is also being considered. When the design of radiation equipment allows it to be used to disinfect large quantities of grain and other products and the costs of such treatment become more competitive, it is likely that ionizing radiation will be used to much greater extent in lieu of chemical insecticides.

Other forms of radiant energy, such as sound and ultrasound, have also been considered for their pest control applicability. They can be used in two main ways: (1) By causing death directly by heating, injury to vital organs, or by applying intense energy levels, and (2) by using lower intensities to which the pests are attracted or their behavior affected. Killing pests directly with high intensity sonic and ultrasonic energy appears impractical because of the cost of producing the required energy and because the energy can only be applied in a restricted area. Artificial sounds and recordings of an insect's own sound have been used to attract insects.

Integrated control techniques.—The joint utilization of several suitable techniques to eradicate pests or manage their population levels is termed integrated control. Integrated control utilizes chemical, biological, and physical techniques either concurrently or in sequence to reduce pests to acceptable levels. Integrated control requires a thorough understanding of the ecology of the pest populations and relies not only on chemical pesticides, but also natural enemies and all other factors in the environment which tend to limit pest populations.

Integrated control is desirable because the flexible nature of the ecosystem allows it to react or adjust to stress in various ways. Thus, to counteract the reactions of the ecosystem or to lessen the impact which causes undesirable side reactions, more than one control method is desirable. For example, rather than applying a large amount of pesticide for the control of Hessian fly on wheat, smaller amounts of pesticide together with delayed seeding and the planting of resistant strains of wheat lessens the flow to the ecosystem, does not produce such a large ecosystem reaction, and controls the Hession fly in a more effective and economical manner than by using pesticides alone.

A similar type of integrated control would be the use of light to attract insects to a bait which had been impregnated with a chemical poison.

The effectiveness of integrated control in pest management is well illustrated with control of the spotted alfalfa aphid. More than a dozen insecticide treatments were initially employed for aphid control. When use of natural enemies and insecticides were judiciously combined, insecticide treatments were reduced to one. However, integrated control involves techniques that may appear quite subtle to the nonecologist, such as special plantings of certain tree types (bald cypress) on reservoir shorelines (*4*). Hence, integrated control is likely to be employed only where knowledgeable personnel are available to initiate and supervise such a program.

In short, integrated control is the manipulation of all the chemical, biological, and physical components of the environment for the control of the pest organism. The proper analysis of the techniques and measures to be employed requires that a systems design be developed for the ecosystem in which the pests are found. Ecosystems contain dynamic interactions, therefore successful management of the system must also be flexible and dynamic. To augment integrated control techniques, fundamentals of ecology, genetics, plant pathology, agronomy, and entomology, as well as system analysis, economics, and biomathematics must be employed.

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MONITORING OF PESTICIDES IN THE ENVIRONMENT

The initiation of specific monitoring programs for pesticides has been the result of the very specialized interest of individual governmental agencies. As described below, the Departments of Agriculture, Interior, and Health, Education, and Welfare all have substantial roles in pesticides monitoring. Even within these agencies, the responsibilities are divided according to the specific missions of the subagencies such as the Food and Drug Administration, the National Air Pollution Control Administration and the Environmental Control

Administration in HEW and the Federal Water Pollution Control Administration and the U.S. Geological Survey in Interior.

Recognizing the proliferation of studies and responsibilities, a Federal Committee on Pest Control was established which is charged with coordination of activities and exchange of information amongst the agencies involved in pesticide management. The Committee has no responsibility for initiating activities. Consequently, there are considerable gaps in the monitoring program with the result that an assessment of the level of pesticides in the biosphere, and how it is changing, is almost impossible to make. For example, among the largest reservoirs for the storage of pesticides being added to the environment are the oceans and yet no agency of the U.S. Government has responsibility for monitoring pesticide levels in the oceans.

Many ad hoc studies of pesticides in the environment are being made by various research agencies, and many of these are being reported in the literature, but there is a need for evaluation of the entire biosphere, the inputs of pesticides to the biosphere, their degradation, translocation, and storage and their rate of accumulation in the various elements of the biosphere. This would help identify elements in the environment that should be monitored, and help indicate priorities for the control of pesticide use.

Amongst the more obvious needs in a monitoring program are the following:

(1) An agency with initiative for instituting monitoring programs where they are necessary. Such an agency would have responsibility for overall assessment of pesticide use.

(2) Studies of pesticide levels in the oceans, groundwaters, lakes, rain, water supplies, waste waters, and in the air.

(3) Studies of pesticides use and levels in the environment outside the United States. The use of pesticides in agriculture in developing countries is only in its infancy. Indiscriminate and excessive use of pesticides, which can be expected because of the technological and educational level in these countries, will virtually assure that considerable fractions of these pesticides will find their way outside the countries of application and into the United States amongst other countries, just as their use in the United States and other industrialized countries has resulted in pesticide accumulations elsewhere in the world.

(4) Assessments of pesticides levels in biota, with special attention to levels in fatty tissues of all species of life. For example, bacteria concentrate pesticides and the accumulation of pesticides in bacteria in all phases of the environment may involve bacteria as a significant storage reservoir.

In addition, there needs to be a large investment in improvement in analytical methods for monitoring pesticides in all environmental media, with emphasis on automated monitoring so that data can be readily stored and amenable to analysis by computer. The continuous analysis of data gathered in a comprehensive monitoring system would reveal points of potential danger and permit regulatory control measures to be instituted before permanent damage is done.

Pesticides in food and feed.—The Federal program for monitoring pesticide residues in food and feed is comprised of: (1) Surveillance programs operated by the Food and Drug Administration, U.S. Department of Health, Education, and Welfare, and (2) pesticide residues studies of meat samples provided by the Livestock Slaughter Inspection Division, Consumer and Marketing Service, U.S. Department of Agriculture. The objective of this program is to determine pesticide levels in unprocessed and commercially processed consumer food commodities, animal feeds and composites of food items prepared for human consumption. Studies include: (1) A continuing market basket study to determine pesticide levels in the basic 2-week diet of a 19-year-old male, statistically the Nation's largest eater, and (2) the nationwide surveillance of unprocessed food and feed.

The Department of Agriculture in 1964 initiated a program specifically designed to establish pesticide residue profiles in limited areas. Pilot studies are being conducted in five areas in the Mississippi River Delta and in Yuma, Ariz., and Grand Forks, N. Dak. Representative farms are selected where records of the kinds and amounts of pesticides used have been kept for 10 to 15 years. The program is designed to determine existing pesticide levels in soils, sediment, water, crops, livestock, and certain species of aquatic and land animals inhabiting the study areas. In addition, soil samples have been analyzed from 17 locations having high pesticide use histories; 13 locations where pesticides were used occasionally such as forests and range lands; and 13 locations on Forest Service lands and national wildlife areas where no pesticides were reported to have been used.

In the surveillance of domestic and imported meat more than 5,000 samples were obtained in 1965 and 1966 from animals slaughtered in federally inspected establishments.

The Food and Drug Administration surveillance program was expanded substantially in 1963. For several years thereafter, FDA collected and examined about 25,000 samples annually, subjecting them to multiresidue methods of analysis using gas-liquid chromatography. Presently, about 9,000 samples are examined annually. Most of these samples are "objective"; that is, not related to any specific suspicion of excessive residue and therefore expected to be representative of the food supply as a whole.

Pesticides in fish and wildlife.—Efforts to determine pesticide levels in fish and wildlife are being carried out by the Bureau of Sport Fisheries and Wildlife, U.S. Department of the Interior. The monitoring of pesticide levels in clams, oysters, and estuarine sediments is a ment of the Interior and the Food and Drug Administration of the Consumer Protection and Environmental Health Service, Department of Health, Education, and Welfare.

The objective of these programs is to determine on a national scale the levels and trends of pesticides in the bodies of selected forms of animals and in estuarine sediments. Fish and wildlife at or near the top of the food chain are being sampled at each sampling location. The species selected are not extremely sensitive to chemicals, are geographically well distributed, reasonably numerous, and easy to collect. Residues in these organisms reflect residues in organisms at lower levels of the food chain. The species chosen for monitoring of pesticides in wildlife include the mallard or black duck, starling, and the bald and golden eagles. The monitoring of pesticide levels in estuaries is done by the determination of pesticide levels in oysters and clams. The upper estuarine sediment is also being sampled.

Pesticides in surface waters.—The program for the monitoring of pesticides in surface waters is being carried out by the FWPCA and Geological Survey of the U.S. Department of the Interior. The purpose of this program is to provide information on the extent of pesticide contamination of the Nation's water resources. Monitoring is currently confined to the surface waters in the major drainage rivers of the United States. Sampling sites have been chosen according to the following criteria: (1) Locations have been selected at or near the mouths of major river drainages throughout the country; (2) other sampling sites on river systems have been chosen when it is believed that a reasonable measure of pesticide contamination cannot be obtained by sampling at the mouth; (3) sites have been selected at or near stream-gaging sites; (4) sites have been located where the quality of river water is now being affected by the use of pesticides; (5) if possible, the locations have been chosen where other kinds of water quality data have been or are being collected; and (6) stations have been located, if possible, at points from which historical data in the form of carbon filter extracts are available. Fifty-three sampling locations have been selected. In general, lakes and ground waters, as well as ocean waters, which constitute the largest reservoir of pesticides, are not being monitored.

Pesticides in soil.—Most of the soil monitoring program has been carried out by the U.S. Department of Agriculture. Other aspects are being conducted by the U.S. Department of Agriculture in cooperation with State and other Federal agencies. The objective of this program is to determine levels of pesticide residues in soils of selected areas in the United States and to detect any significant changes in these levels. Soil monitoring sites were chosen where possible to coincide with sampling sites of other agencies in the Federal pesticide monitoring network so that soil data may be correlated with pesticide levels in other environmental media. Soils in areas of high pesticide usage as well as areas of low usage are being monitored.

Pesticides in air.—An air monitoring network, eventually to include pesticides, is being established by the National Air Pollution Control Administration. Because of the considerable variability of pesticides in air, their fallout and washout and the uncertan patterns of air in air, their fallout and washout and the uncertain patterns of air movement over the earth, the results of such monitoring will require sophisticated interpretation.

Pesticides in people.—The monitoring of pesticides in people is being carried out by the Division of Community Studies of the Food and Drug Administration, CPEHS, HEW, at the Communicable Disease Center in Atlanta. The purpose of this program is to determine on a national scale the levels and trends of some of the commonly used pesticides, both in the general population and in population segments where increased exposure levels are known or suspected. The present monitoring program hopes to provide statistically and epidemiologically sound information for use in the evaluation of the significance of man's total exposure to pesticides. Such items as geographic area, conditions of exposure, type of pesticide, sex, and body tissue samples are taken into account and evaluated. Two types of monitoring studies are being conducted—a limited national survey of the general population and an indepth study of selected communities in high-use areas. Three population groups are being sampled: (1) Occupationally exposed workers (agricultural applicators, workers in pesticide formulating plants, pest control operators, greenhouse workers and aerial spray pilots); (2) individuals not occupationally exposed but known to be repeatedly exposed (people living in agricultural areas); and (3) the general urban population (people whose exposures are largely limited to pesticide traces in food, water and air). With long-term epidemiological data, it may be possible to distinguish the effects of continuous low levels of exposure to pesticides on human populations.

Analytical methods.—The accurate measurement of environmental pesticide levels requires the continuing development of analytical methods sensitive enough to measure the low concentrations present. The instrumentation, its correct operation and analysis of the results obtained by these techniques require highly trained and experienced personnel. Therefore, the proper monitoring of the pesticide levels in such major environmntal components as food, air, and water is a highly sophisticated operation.

Less complicated techniques are available but suffer from a lower sensitivity. If insensitive procedures are adopted to save costs for some monitoring operations, the use of a highly toxic compound could be approved because of the poor method of analysis used, while the use of another, less toxic compound is denied because a sensitive method of analysis is available that indicates the presence of an infinitesmal amount. Clearly, in order to measure environmental pesticide levels and evaluate their effects meaningfully, the most modern and sensitive techniques available must be employed regardless of the cost and complexity involved.

The measurement of pesticide residues in food is perhaps the most advanced of all such techniques. The time required per analysis is relatively low, the differentiation of the many different pesticide chemicals may be reliably carried out, and the sensitivity is high, allowing the accurate measurement of concentrations well below established tolerance limits. These techniques are also capable of determining accurately a number of significant metabolic or alteration products. Equipment of relatively high initial cost is required, however, and highly qualified personnel must perform the analyses and interpret the results.

The technique of measuring pesticide levels in air is not as highly developed as those for food and water. The measurements are most generally accomplished by passing a known volume of air through an organic solvent contained in a gas scrubbing device. Ethylene glycol is used most generally as the solvent. Care must be taken to obtain a reagent blank free of compounds which would interfere with either the electron capture or flame ionization gas liquid chromatography systems. The presence in the atmosphere of so many compounds in small concentrations requires that the analyst be certain that all the peaks produced originated from the scrubbed air.

These compounds also present problems in identification and differentiation amongst pesticides and other organic compounds, both synthetic and naturally produced, that are found in the environment. Competent analytical chemists capable of interpreting environmental polution data must be employed.

Continuously operating monitoring systems are desirable because of the large amount of air which may be sampled, but such systems require an inline calibration in order to obtain accurate measurements. These are expensive to acquire and operate.

The sensitivity of commonly used procedures is as low as 0.1 p.p.b. and is limited by the volume of air sampled and the cleanup efficiency. In general, the sensitivity of the methods now in use is not adequate for the proper evaluation of pesticide levels in urban areas.

The two most commonly used procedures for extracting pesticide residues from water are the batch method and the continuous extraction method. In the batch procedure, a definite volume of water, usually 1 liter, is extracted with an organic solvent such as chloroform, hexane, etc. The continuous extraction method involves the passage of a large volume of water through an extraction apparatus. The carbon absorption method of extraction whereby pesticides are absorbed onto activated carbon and then extracted has not proven to be of sufficient sensitivity so is not used to any great extent at the present time.

Using the parameters described in the Food and Drug Administration Pesticide Analytical Manual, as little as 1 p.p.b. heptachlor epoxide, for example, can be measured by the batch technique. The sensitivity of this method may be improved at the expense of system stability. The sensitivity of the continuous extraction procedure is limited by the volume of water extracted. Once again, trained analytical chemists are required to interpret the data.

Analytical Needs.—In addition to refining existing methods and developing new methods where no suitable procedures are now available, research leading to the development of automated instrumentation is urgently needed. This report identifies the many sectors of the environment that are not now being adequately monitored, and proposes extensive new investigations to identify epidemiological and other hazards in the environment. The implementation of the recommendations will require a considerably greater investment in pesticide analysis. In the long run, the most economical approach to these massive operations will have to be through automation.

Systems Analysis of Pesticides in the Environment

Guides to policy decisions for institutional management of pesticides in the environment require the continuous assessment of (1) the rates of input of the various pesticides into the environment; (2) their various rates of degradation and formation of toxic degradation products and in turn the rates of degradation of these products; (3) the mechanisms and rates of translocation of pesticides among the several media of the environment; (4) and the storage and rates of accumulation of pesticides in the various elements of the biosphere. The concurrent examination of these changing rates and amounts constitutes a "systems analysis".

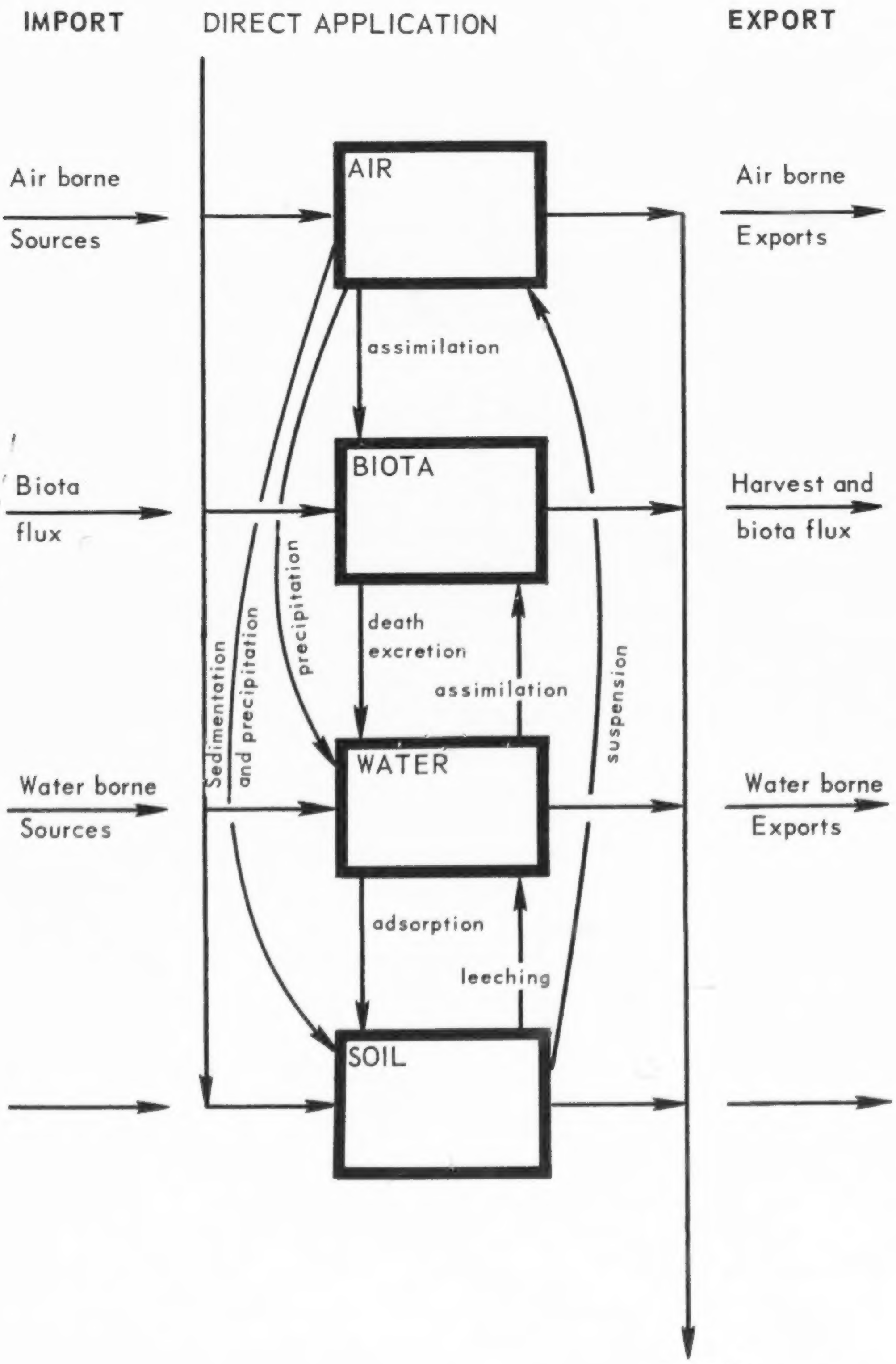
Such systems analysis research would have several objectives: It would identify the data that need to be obtained to permit the analysis to be made; it would permit an evaluation of the trend of accumulation of pesticides in the environment and it would enable a cost-benefit analysis to be made that could serve as a guide to pesticide utilization.

The first step would be to establish a conceptual systems analytic framework for the evaluation of interregional and intraregional movements of persistent pesticides as they are dispersed into the environment, while the second step would be to develop an operational regional model within the United States for the movement of persistent pesticides, including the import and export of residues into and out of the region. While the construction of a global model would be a desirable goal, operationally the task is not a feasible one under reasonable limitations of time, effort, money, and data availability. Empirically derived inferences can be drawn from a regional model that perhaps could limit the added value of a global model.

An essential part of the research, and possibly one of its most valuable contributions, would be an assessment of the adequacy of current research and data collection activities upon which such models will be built and upon which policy decisions must be made. The value of such data and research can be measured only in terms of uses to which they can be put.

The attached schematic diagram depicts a regional system for pesticides movement among the air, biological, water and soil storage and transport media. Also represented are additions of pesticides through imports from other regions and direct application within the system while losses are represented by export and decay. By constructing a mathematical model of the regional system using established relationships among its components and calibrating the model with the best available estimates for model parameters, it is possible to assess the impact on system response of alternative policies relating to rates and methods of application. System response, measured by the amounts of pesticides in any component at any time, can be translated into economic, health, and ecological consequences, some of which have previously been established.

Because pesticides include a wide variety of chemicals which are applied and dispersed by a number of processes and because persistent pesticides are of primary concern in protecting environmental quality, the research might initially be directed toward the behavior of the persistent pesticides, the chlorinated hydrocarbons, with the other pesticides being introduced after the model has been designed and verified.


IMPORT
DIRECT APPLICATION
EXPORT
Air borne
Sources
AIR
Air borne
Exports
assimilation
Biota
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BIOTA
Harvest and
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Sedimentation
and precipitation
precipitation
death
excretion
assimilation
suspension
Water borne
Sources
WATER
Water borne
Exports
adsorption
leeching
SOIL
DECAY

Flow diagram for regional system for pesticide movement in the environment.

CHAPTER 3

Effect of Pesticides on Nontarget Organisms Other Than Man

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EFFECTS ON NONTARGET ORGANISMS OTHER THAN MAN

SUMMARY AND CONCLUSIONS

Man is an integral part of the living system, which includes about 200,000 species in the United States. Most of these are considered to be essential to the well-being of man. Pesticides are now affecting individuals, populations, and communities of natural organisms. Some, especially the persistent insecticidal chemicals such as DDT, have reduced the reproduction and survival of nontarget species.

Pesticides are dispersed via air, water, and the movements of organisms. The most significant concentrations are found in and near the areas of intensive use, but traces have been found in the Antarctic and other areas far from application. Pesticides have reduced the populations of several wild species. Both extensive field data and the results of excellent controlled experiments demonstrate that certain birds, fishes, and insects are especially vulnerable. There are suggestions that pesticides in the environment may adversely affect processes as fundamental to the biosphere as photosynthesis in the oceans.

However, the scarcity of information concerning the influences of pesticides on natural populations prevents adequate assessment of their total effects. Less than 1 percent of the species in the United States have been studied in this connection, and very few of these have been subjected to adequate observation. Present methods and programs for determining the influences of pesticides on nontarget organisms are inadequate. Little data exists on the distribution, location, and impact of various pest control chemicals in the natural living systems of the world.

The general nature of the effects of pesticides on nontarget species populations and communities can now be suggested. Although there is usually greater similarity of reaction between closely related species, each species reacts differently to specific pesticides. DDT, for example, causes egg shell thinning in ducks and falcons, but not in pheasants and quail. Pesticides from the air, water, and soil may be concentrated in the bodies of organisms. The concentrating effect is frequently enhanced as one species feeds on another and passes the pesticide from one link to another in the food chain. Hence, predators like some birds and fish may be exposed to levels several thousand times the concentration in the physical environment. Some nontarget organisms can, under highly selective pressure from pesticides, evolve resistance to them. The surviving resistant individuals may pass extremely high concentrations to their predators. In communities exposed to pesticides, the total number of species is usually reduced and the stability of populations within the community is upset. Often, beneficial species are unintentionally eliminated. Such a reduction in the number of species is frequently followed by outbreaks or population explosions in some of the surviving species, usually those in the lower parts of the food chain. When a vital link low in the food chain is eliminated, many predators and parasites higher in the food chain are often also destroyed.

The Committee has reached the following conclusions:

1. Adequate methods should be developed and utilized for evaluation of the hidden costs of the uses of pesticides.

> Such evaluation is essential as part of the development of useful estimates of all of the benefits and costs to society. Some partial estimates of the direct benefits are available and useful. Adequate data are not available on such indirect costs as losses of useful fish and wildlife, damage to other species, and any esthetic effects. These are required to guide rational decisions on the proper uses and control of pesticides so that the net gains will be as great as possible while the net losses are minimal.

2. Persistent chlorinated hydrocarbons which have a broad spectrum of biological effects, including DDT, DDD, aldrin, chlordane, dieldrin, endrin, heptachlor, and toxaphene, should be progressively removed from general use over the next 2 years.

> These pesticides are causing serious damage to certain birds, fish, and other nontarget species among world populations. Some of these species are useful to man for food or recreation, some are essential to the biological systems of which he is a part, and some merit special protection because they are already endangered.
>
> These pesticides have value in specific circumstances, and we suggest that they be used only under license and with special permits. The system for assuring this careful use should be established as the unrestricted use of these materials is phased out over the 2-year period.

3. The release of biocidal materials into the environment should be drastically reduced.

> In addition to restriction of the use of hazardous pesticides, many techniques can be applied which will minimize the re-

lease of pest control chemicals. In industry, improved chemical and engineering processes could reduce the quantity of contaminated wash water; more effective methods can be developed for disposal of unused stocks and residues of pesticides; and improved surveillance of effluents would be desirable. For home use, improved materials and methods of application can be created and employed with greater discretion on the part of the individuals involved. For large-scale applications, conversion to integrated methods of pest control, care in the selection and application of specific chemicals, and preference for short-lived pesticides would reduce release to the environment.

These efforts, combined with increased research and education, would slowly but effectively reduce the damage to nontarget species.

4. The U. S. Department of Health, Education, and Welfare or another Federal agency should negotiate a contract with a suitable national professional organization to develop a system, complete with standards of training, testing, and enforcement, for the effective restriction of use of selected pesticides known to be especially hazardous to man or to elements of the environment.

To achieve an adequate and prompt further reduction in the use of certain pesticides and still permit their use where no adequate substitute is acceptable, there must be a system of regulation based upon State or local authority but using uniform national standards. This system should provide for use of the selected pesticides only by or under the immediate supervision of a licensed operator meeting certain standards of training, competence, and ethics.

5. Educational efforts relating to the proper and improper usages of pesticides should be improved and expanded.

The most important element in the wise use of pesticides is the individual person who selects the chemical to be used and decides upon the methods of application. Suggestions have been provided elsewhere for the proper training of all large-scale applicators. It is equally important that homeowners, gardeners, students, legislators, civic officials, and others receive adequate and correct information and develop proper attitudes. Such education could contribute greatly to wise use of pesticides, and also to rational response to governmental efforts to protect public health and welfare while gaining as much advantage as possible from pest control methods.

6. All pertinent Federal and State agencies should review and improve policies and practices of pesticide use.

The beneficial uses of pesticides have been accompanied by a wide variety of policies and practices which have sometimes been wasteful, unnecessarily destructive, or ineffective. We offer the following suggestions to be included among the guidelines for wise use of pesticides:

a. Pesticides should be applied only when there is evidence that pest densities will reach a significant damage threshold.

b. Effective pest control does not usually require eradication of the pest species, and should be directed toward optimal management of pest densities.

c. Support for research and demonstrations should be provided to projects based on the systems approach to pest management and control.

d. Diversity of species is biologically desirable since it contributes to the stability and efficiency of life systems.

e. No species should be eradicated except as a carefully selected pest and when compensating human gains are ecologically sound and clearly established.

f. Special care must be taken to prevent any damage to the species and mechanisms which are of fundamental importance to biological systems. For example, oceanic photoplankton produces most of the oxygen necessary for the earth's biological system.

g. Requirements for food quality should not be so high as to require excessive use of pesticides. Customer preference, and regulatory requirements, for unblemished fruit and vegetables and the complete absence of insect parts have encouraged heavy use of pesticides.

h. New pesticides should be given interim approval which permits contained use in limited but typical circumstances prior to general approval. The pattern of careful progressive risks would encourage new developments without endangering the public interest.

i. Effective incentives should be established to encourage the development of improved pest control techniques. The cost of entering a new product or testing a different control technique is high. Since effects on the national welfare are involved, proper governmental encouragement of private industrial efforts may be appropriate.

7. Registration requirements should be strengthened and redesigned to permit initial provisional approval, then general use approval, and to require periodic review and re-registration of materials.

> Registration of pesticides offers the most important opportunity for estimating potential benefits and costs in advance of wide usage. In addition to present registration application information, useful estimates should be provided of the persistence of the pesticide, on the breadth of its biological impact, and on its fate. These will disclose the nature and possible magnitude of the nontarget effects. If approval is appropriate, we suggest that it be for a short-term period and for use under defined circumstances where risks are confined, and that general use be considered after such field experience. Since some of the significant effects in nontarget species are subtle, sublethal, and difficult to detect, we recommend that all pesticides be subject to periodic review and approval.

8. All commercial applications and other large-scale applications of pesticides should be performed under the supervision of competent trained persons.

> The complex responsibilities of pesticide application involve both achievement of the greatest possible benefit and maximum prevention of damage. These require considerable knowledge of the management of crops, the biology of desirable and undesirable species, the effects of weather, and the effects of biocide in the ecosystems. They also require application of professional judgment and use of professional standards of conduct and responsibility. We suggest that all such applicators should be properly trained, required to demonstrate their competence, and awarded evidence of their ability. Incentives in the forms of salary and recognition will be needed to encourage such professional training.
>
> Training programs for pest management specialists of all types, including applicators, should include the concepts of systems approaches to pest control and emphasize the relationships between pest management activities and the total biological community affected.
>
> Since new information is emerging rapidly in pest management, refresher courses for county agricultural agents, applicators and others involved in the uses of pesticides and other control techniques would be of special value.

9. The production of additional information and comprehension should be encouraged and supported on many aspects of pesticide use and effects.

Experience with pesticides has revealed many serious gaps in available knowledge. Research is urgently needed on many general and specific problems. The following problems are all related to nontarget effects of pesticides, and many of them are also pertinent to other areas of pesticide use, to successful management of animals and plants, and to fundamental science.

a. What are the acute effects of the common pesticides when used on the many species of wildlife and other organisms which may be exposed to them?

b. What are the effects of indirect and chronic exposure?

c. What is the nature and magnitude of the effects of insecticides on beneficial insects and other species?

d. What are the normal patterns and variations in natural biotic communities, as baselines for understanding future pesticide pollution effects?

e. What mechanisms exert natural control on various pest populations?

f. How can we best estimate pest populations and predict their trends?

g. What are the full potentials and realistic limitations of the pest control methods which are suggested as alternatives to chemical pesticides, including predators, parasites, pathogens, cultural control, sterilization, attractants, repellants, genetic manipulation, and integrated approaches?

h. What improvements are possible for pesticide packaging and disposal (including degradable containers) to minimize threats to nontarget species?

10. A vigorous specific program should be created to bring the 100 most serious insect pest species of the United States under optimal control.

These require about 80 percent of the insecticides now in use. Dramatic focusing of attention on the "100 worst" could lead to rapid improvement in the species-specific insecticides, biological control methods, or integrated control programs.

11. The responsibilities of the several Federal agencies involved in pesticide regulation and control must be more clearly defined and certain specific activities should be improved or initiated by appropriate agencies.

Procedures and patterns for the regulation and control of pesticide use have emerged during the last 30 years in response to changes in law, evolving practices in agriculture, production of new chemical materials, changing public concern with health effects and nontarget damage, emerging scientific comprehension of benefits and costs, and other unstructured events. Both benefits and costs are now so large as to merit the national allocation of responsibilities. We suggest careful review and reassignment, by law if necessary, of the proper role of—

a. The Department of Interior, charged with protection and enhancement of nonagricultural resources and with water quality control.

b. The Department of Agriculture, charged with assisting in the maximum production of food, fibers, and other culturable crops in ways which are not detrimental to other interests.

c. The Department of Health, Education, and Welfare, charged with protection and improvement of human health and welfare.

d. The National Science Foundation, responsible for improved comprehension of fundamental processes and assisting in their application for human benefit.

e. The Environmental Quality Council, Federal Committee on Pest Control, and other coordinating agencies.

Other agencies are, of course, involved as users of pesticides and in other functions. Those listed above, however, appear to comprise the areas of primary attention. In addition to present programs and activities related to pesticides, we suggest the following services for new or additional emphasis:

a. A taxonomic and identification service should be established to provide increased knowledge and reference standards for biological investigations related to all fields of pest control.

b. Broader monitoring should be undertaken of the types and quantities of pesticide transmitted by various means and reaching nontarget species. Bioaccumulators like oysters and other molluscs can be unusually useful as indicators, and the levels of concentrations in predatory species are of special importance.

c. Early indications of undesirable effects must be detected effectively and followed by appropriate action. When the early warning system suggests a potential pollution hazard in the environment, the acquisition of additional pertinent information by the scientific community should be supported.

d. Multidisciplinary investigations of alternative control techniques should be carried out whenever present control methods are shown to contain potential hazards.

e. A single agency should assume the responsibility for assimilating information on the effects of pesticides on nontarget species and transmitting it to appropriate regulatory and educational centers.

f. Measurable predictors of potential hazards from pesticide use should be agreed upon and might be made the basis of a handicap tax to be applied to each pesticide in proportion to its pollution hazard.

INTRODUCTION

The earth supports a complex system of living organisms of which man is an integral part. The system also involves the chemical and physical environments of the earth's crust, the oceans, the atmosphere, and the interfaces between them. In these environments, millions of kinds of organisms have evolved, each species with a specific set of requirements.

Certain basic ecological principles are well established. Only the plants can synthesize organic compounds, through photosynthesis. Some animals feed on plants, some on other animals, and some on both. Bacteria and a few other organisms participate in the breakdown processes which return the chemical elements to simple forms which can be reused and recycled. This master cycling is accompanied by lesser cycling of specific elements and compounds in ways which are only partially understood. Events on land, in the sea, and in the air can affect the processes and rates over a very wide area of the earth, since this is a single global life system.

Only in recent centuries has the human species had more than trivial impact on biological events in the world. In the century since the industrial revolution, man has brought large areas under control, changed populations and, eventually, collected and used such large quantities of some materials as to modify parts of the biosphere. Improved agricultural yields and grave pollution problems are both aspects of these accomplishments.

THE FOOD NETWORK

For the present discussion, the significant questions are whether or not man's uses of chemicals to kill or control pest species at specific sites has caused serious damage to nontarget species, populations, and processes in the world life system.

Solar energy captured by photosynthesis is available for biological use by herbivores, then (in quantities which rapidly decline) to omnivores, carnivores, supercarnivores, and organisms of decay. This sequence is easily stated, but rarely occurs in nature. There, the energy transfer process is more accurately described as a network, since different stages of each species play differing roles and since an enormous number of species are involved.

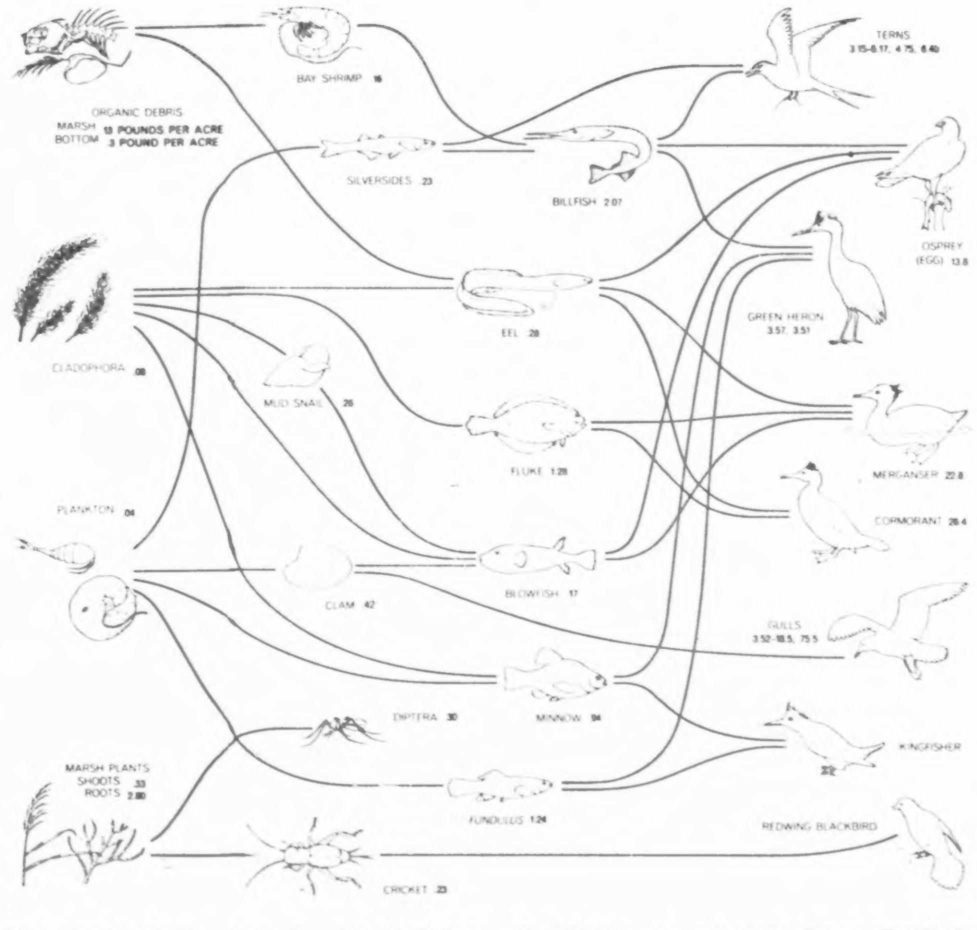
When a persisting chemical compound is made available to this biological network, its movements and biological effects may involve many species, locations, and degrees of effect. So it is with some of the persistent pesticides. Among the benefits the world has received from the use of DDT, high value should be placed on the increased knowledge and appreciation which has been gained of the global unity of the biological network and, therefore, of the global effects of local chemical usages.

Such chemicals are directed toward a pest species. Release places the chemical on or in the target and, invariably, on or in other species and parts of the environment. The chemical begins its movement through the ecosystem. We cannot quantitatively describe the transport of DDT or other chemicals, except for a short period in a river or pond, but evidence is sufficient to prove the constant movement of part of the material into and through living organisms. There, the chain effect of feeding patterns may create increasing concentrations in subsequent food levels. In general terms, only about 10 percent of the energy in one trophic level will be transferred to the next level, and the rest will be used for respiration or released as wastes. Chemicals which are preferentially absorbed into living organisms and stored for extended period, as are DDT and its derivatives, may, therefore, be concentrated greatly up the food chain. At any point, the chemical may reach a species which is susceptible to it and damage will result. The nature of the food web and the distribution of a pesticide are partially illustrated in figure 1, from the article in *The Scientific American* by A. M. Woodwell.

If release of such a chemical is terminated, the quantity in the ecosystem will gradually decline in accordance with the rate of natural conversion of the substances to compounds or elements which do not have the same effects. This decline in biological organisms will occur over a long period, related to the length of life of various involved species and many other factors. Eventually, the amount of chemical compound will be insignificant in the ecosystem.

SPECIES AND SPECIFICITY

Since the appearance of the first organism on earth some 4,000 million years ago, about 99 percent of all species have become extinct. Gradually, species after species has been replaced by those better adapted to the new environmental conditions which also have been continually changing. The evolutionary process and the development of new species take thousands of years; a slow process and especially


ORGANIC DEBRIS
MARSH 13 POUNDS PER ACRE
BOTTOM 3 POUND PER ACRE
BAY SHRIMP .16
SILVERSIDES .23
BILLFISH 2.07
TERNS
3.15-6.17, 4.75, 6.40
OSPREY
(EGG) 13.8
CLADOPHORA .08
EEL .28
GREEN HERON
3.57, 3.51
MUD SNAIL .26
FLUKE 1.28
MERGANSER 22.8
CORMORANT 26.4
PLANKTON .04
CLAM .42
BLOWFISH .17
GULLS
3.52-18.5, 75.5
DIPTERA .30
MINNOW .94
KINGFISHER
MARSH PLANTS
SHOOTS .33
ROOTS 2.80
FUNDULUS 1.24
REDWING BLACKBIRD
CRICKET .23

The food web is a complex network through which energy passes from plants to herbivores and on to carnivores within a biological community. This web showing some of the plants and animals in a Long Island estuary and along the nearby shore was developed by Dennis Puleston of the Brookhaven National Laboratory. Numbers indicate residues of DDT and its derivatives (in parts per million, wet weight, whole-body basis) found in the course of a study made by the author with Charles F. Wurster, Jr., and Peter A. Issacson.

Permission to use granted by The Scientific American. *From Woodwell, G. M.: Toxic Substances and Ecological Cycles. The Scientific American. 216: Vol. 216, March 1967.*

slow relative to man's life span. Today an estimated 2 million plant and animal species exist on earth. Most of these species are found in the favorable tropical regions with fewer species existing in the temperate regions and only a few able to endure the harsh polar climates.

No one knows how many of these estimated 2 million species are necessary in man's environment for his survival and welfare. Certainly he cannot survive with only his crop plants and domesticated animals. Most natural species interact in maintaining a functional life system. Understanding the biology of the life system is most difficult because of vast numbers of species and the complexity of their interactions. Current knowledge suggests great caution in evaluating the worth of a particular species.

There is a pattern of basic characteristics common to all organisms, including a genetic code, a reproductive system and a structure primarily dependent upon carbon, oxygen, hydrogen, and nitrogen. These characteristics plus the basic requirements of life (energy, matter, and a suitable environment) form a set of similarities which are applicable to all forms of life.

The study of plant and animal classification has revealed both similarities and specificity in organisms. Each species is a unique biochemical entity; therefore, species react to each pesticide in a different manner.

The ancestral system of life and the various lineages provide a recognized organization and pattern for life in general and species groups in particular. The more similar the species group, the more likely its members will respond to environmental factors in a like manner.

PESTICIDES AND TOXICITY

Approximately 900 chemicals are registered with the USDA–PRD as pesticides for use against about 2,000 pest species. The estimated 200,000 nontarget species in the United States respond in diverse ways to the toxicity of these chemicals. In some cases, little or no permanent harm is done as with many of the chemical repellents and attractants. With other pesticides, however, the effect upon nontarget organisms may be as severe (in some cases with DDT) as against the target organisms. Of vital consideration is the fact that the vulnerable nontarget organisms are often of value to this particular ecosystem.

In general, each poison has more than one mode of action; however, in most cases the modes of actions of the pesticide against target organisms are only partially known. Even less is known about the actions in nontarget organisms. Even if the target pathophysiology of a chemical were known, its effect on nontarget organisms may be quite different due to the differences between species. For example, DDT, DDE, and DDD are thought to act as a nerve poison on most insects, but in some birds DDE especially influences their physiology of egg production, and causes thin egg shells.

Until the mode of pesticide action is completely known and the differing responses of species and individuals are understood, it will continue to be impossible to generalize on either the pathophysiology or population effects of pesticides on other target and nontarget species.

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ROUTES OF EXPOSURE

This section of the report will deal with various aspects of manufacturing, transportation, and distribution of pesticides wherein leaks, spills, and waste disposal are potential, and sometimes documented, sources of environmental pollution; hence, exposure of nontarget organisms.

Manufacturing of the active ingredient.

Any leakage which occurs may or may not be intercepted by "fail-safe" effective traps. This "leakage" is usually slow and the contribution of pollution to air or water may be of an inconsequential magnitude; but with today's technology, detection systems can be designed to monitor such losses. Materials which are toxic, or less degradable materials which tend to accumulate in food chains, require and generally receive more rigorous surveillance and control so that leakage, if not eliminated, is trapped or impounded for subsequent waste treatment procedures.

There is a greater pollution hazard when manufacturing requires *direct* contact between processing water and the pollutant because much of this processing water returns to the stream. If the intervening steps (extraction, neutralization, decomposition, biodegradation, adsorption, etc.) for removal of contaminants from the water are inadequate to lower the pollutant to an acceptable level, then this waste water is troublesome and expensive to deal with (see waste disposal paragraph below). Improved processes and engineering systems which require the use of less water for washing extraction and cooling are being developed for modern-day plants.

Accidental spills or sudden releases of contamination to the water or air contrast with leakage which is slow, continuous and less obvious. Methods of interception are important to contain and confine hazardous materials in the event of an accidental release or spill. Interception systems may be simple and obvious, such as dikes around storage tanks or separate sewerlines leading to special impoundments. In some cases elaborate systems may be installed to prevent escape of contaminated liquid, dust, or vapor from enclosed buildings. The relative hazard presented by the materials must be a prime consideration in the design and operation of any manufacturing plant.

Formulating plants are confined largely to mixing, blending, and packaging operations and are generally less complex than manufac-

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turing plants; because of this, less demanding of skill and sophistication. Leaks and spills are as likely, and perhaps more likely, than from manufacturing plants but the total quantity which potentially could be released to the environment at one time would be less than a similar accidental release from a primary manufacturing plant. There are more formulating plants, less specialized and handling smaller volumes compared to manufacturing plants where "economy of scale" permits large and specialized operations. Chemical and biological waste disposal techniques have proven successful for a large variety of compounds; others are more resistant to degradation. Incineration of solid and heavy liquid wastes is a procedure now being used more extensively. Effluent stack gases are further scrubbed to remove troublesome vapors. Deep well disposal of waste is a method gaining in popularity, but this method is not without substantial risks. A thorough knowledge of the geological structure and the nature of material to be pumped into the well is essential to avoid escape of these wastes from the intended strata.

Transportation of the Pesticide or Formulations Thereof

Environmental pollution which occurs during transportation results from defective packages or from rupture and consequent spills. Design of the package adequate to survive rough handling during transport is an important way to minimize this type of accidental pollution. Corrosion also leads to failure of the container, particularly when long periods of storage are involved. This can be prevented by selection of a suitable container but the costs are higher. Serious instances of spills and leaks during transportation have been reported.

Market Distribution

Leaks and rupture of packages while in the stocks of distributors and dealers are directly related to package design and care in handling. Generally the incidents that happen while in the hands of distributors or dealers contribute little to the overall contamination of the environment. Obsolete stocks can range from small packages sold in hardware stores and garden centers to drum quantities sold by distributors and dealers.

Disposal of old stocks and disposal of used but dreg-laden containers does present a practical problem which requires better practices. Stocks or containers indiscriminately discarded constitute an acute toxicity hazard to the people nearby and also serve as a source of pollution as rain washes any liberated pesticides into the drainage water. Few, if any, communities have adequate means of destroying *large* quantities of toxic materials in municipal waste disposal systems. For the small quantities of pesticides involved in wastes from urban homeowner use including those adhering to empty containers, burial in an approved sanitary landfill appears to offer reasonable protection to nontarget organisms. If wrapped, to discourage animals or children from removing them, and then placed in trash cans for collection by municipal authorities, they should find their way into incinerators or suitable landfills. Pesticides should not be flushed down sanitary or storm sewers.

Under rural conditions, burial away from water supplies is the best available method to dispose of such waste and small used containers. Larger containers can be handled by professional drum reconditioners Improved methods of waste and container disposal are deserving of increased emphasis and support.

Classes of Applicators

1. Homeowners and amateur gardeners purchase small packages and are largely instructed through labels, leaflets, books and articles, and assistance from the dealer who sells the product. The extension service as well as State agencies conduct programs to instruct the homeowner but it is difficult to instruct this class of user in a meaningful way. The large majority of homeowners are not discriminating in the selection and use of pesticides. It is difficult and expensive for both the manufacturers and dealers to educate the homeowner beyond the most rudimentary aspects of proper use and disposal of unused materials. Convenience in application requires special packaging and application devices, and these, coupled with formulation techniques, can lead to more sparing use of pesticides. The homeowner needs materials which cannot pose a threat to his safety nor introduce harmful pollutants into the environment.

The typical urban housing development is not a natural environment for any major part of the biota and thus any damage done by the use of pesticides is local in nature. The same may not be true in larger estates or even suburban residential areas of relatively low housing density. In such situations, the total effect of pest control activities by many homeowners may have a significant effect on birds and soil organisms. Use of weedkillers can cause significant damage to neighboring sensitive plants.

2. Ornamental trees deserve special mention. It is difficult for the homeowner to do an effective job of spraying a large tree. Usually a commercial spray service will be employed. Whether around the home, or around muncipal parks and streets, trees are prized, and especially when they are threatened. Budgets for preventive measures

are usually restricted, hence low cost pest control is stressed. The combined pressures of citizen concern, costs, and urgency to save the trees often promotes overuse of less costly and more persistent materials, particularly DDT. Golf courses receive intensive application of pesticides, on greens particularly.

3. Pest control as it applies to structures (termites, ants, etc.), fumigation and sanitation in manufacturing plants, and specific pest control problems—generally in urban environments—is practiced by a trade generally known as pest control operators. The successful ones are usually knowledgeable and in many cases licensed under municipal or State laws. This class of applicator is in a good position to understand the economics, limitations and hazards sufficient to select and use pesticides properly and minimize environmental pollution. Structural pest control, if done by well-trained and careful operators, is not apt to expose the natural biota because pesticides are directed to areas in and adjacent to buildings where little natural wildlife is found. As always, the disposal of used containers and waste mixes presents a problem. Leaching or erosion of soil treated for termite control may present a hazard to surface and ground water. Recognized and accepted practices to minimize this hazard have been developed.

4. In 1964, 42 percent of all pesticides produced in the United States that year were used in agriculture. The remainder went for export and for domestic nonagricultural purposes. Of the agricultural use, 93 percent of the volume was used in treating crops, 3 percent for livestock, and 4 percent for other agricultural use. Many large agricultural operators employ contract applicators and aerial sprayers. Others purchase specialized equipment for more effective and economical application. Smaller growers vary widely in their practices.

Use on farms, including orchards, is commonly considered to be the most important source of pollution of the soil. Much farm use involves soil treatment or application by coarse sprays, but there is also a large amount by aerial application, mist spray, or dust. Determinations of spray patterns have indicated that only about half of the pesticides applied by air or by mist sprays reach the soil directly under the application. Some of the remainder is retained on plants for variable periods of time before it reaches the soil as residues on foliage. There is evidence of drift of herbicides for several miles and there is no satisfying data on how much becomes relatively permanently airborne. Neither is there any information on how much reaches the air through vaporization, "codistillation," or wind erosion of soil, though it has been speculated that all three methods may be important. The relative importance of farm uses as sources of contamination of air and water remains largely speculative based upon volume of use and some meager evidence of the relative contribution of pollution to streams flowing through farmland and urban areas.

Livestock and poultry raisers use a variety of pesticides. Pollution of the environment from this source contributes little pollution burden with one possible exception, that of dipping vats or spray tanks. When these vats or tanks are emptied and cleaned they have been known to contribute harmful concentrations of pesticides into streams.

Many of the forest areas of the United States are managed under Federal supervision. Large tracts, however, are privately owned. In either case, the management of forest pests has involved widespread applications of pesticides and documented cases of environmental pollution. Control of pests of shade and ornamental trees as well as those of forests is a rather direct hazard to birds and related forest biota. Direct hazards to the aquatic environment usually are minimized by avoiding lakes and larger streams. It is impractical to avoid small streams and there is always the possibility of drift and surface erosion. Ordinarily such applications are not necessary on an annual basis so there is some opportunity of recovery of natural biota between applications, depending, of course, on the persistence of the pesticide and the frequency of application. Economic constraints are severe and aerial application is required for widespread insect control.

5. State, Federal, and other governmental pest programs conducted for control or quarantine purposes are directed at destructive agricultural and forest pests. In addition, pesticide applications are made to control disease-carrying insect vectors, particularly mosquitoes (as for yellow fever, encephalitis, and malaria). If these are applied only as residual treatment of interior surfaces, little environmental contamination results. Insect abatement districts are commonly established on a county or regional basis in order to reduce the nuisance of mosquitoes. Noxious weeds are also the object of governmental control programs. Economic constraints are a major factor in the choice of methods and materials. Larvicidal treatments for mosquito control require direct treatment of water with the pesticides. If the materials used are persistent (which favors effective mosquito control and less cost), there is a high probability of pollution of the environment and exposure of nontarget aquatic organisms.

Organized mosquito control involves two very distinctive approaches: larval control and adult control. With the exception of domestic mosquitoes such as *Aedes aegypti*, larval control presents hazards to certain nontarget organisms in the aquatic environment whether it is done by the application of insecticides, the control of weeds by herbicides, or even by water management by drainage or level control. With each of these techniques, the safeguards lie in the careful selection of chemicals, control of dosage, and timing of operations. Evening or nighttime operations minimize exposure to pollinating insects and to birds. Fogging or mist sprays for adults depend upon small particle size which may increase the contamination of air.

Governmentally sponsored control operations to prevent the spread of pests or to eradicate infestations under certain conditions often result in heavy rates of application of pesticides in an effort to achieve 100 percent control. Ideally, such applications are pinpointed to specific areas of infestation and are restricted to one or a very few applications, both of which tend to localize the exposure of natural biota. Large-scale agricultural or community health programs, on the other hand, tend to involve larger areas at a specific time than do private control operations.

6. A growing volume of pesticide is being applied by commercial applicators who are hired to make applications for home owners, growers, governmental units, road commissions, etc. Aerial application of insecticides, brush control along utility rights of way, weed control along roads and railroads, mosquito control and forest insect control are examples of spray applications commonly made. In urban areas commercial sprayers perform this service for homeowners including mosquito control, ornamental pest control, weed control in lawns, and a variety of other procedures around the premises.

7. The final class of applicator mentioned here is associated with a manufacturing or treatment plant wherein the pesticide is deliberately applied to the processed article in order to prevent subsequent attack by insects, rodents, molds, bacteria, marine organisms, etc. This may involve fabric treatment (moth, or mold proofing), wood treatment (insects, fungus, marine borers), preservatives and antifoulants for paints and coatings (marine paints included). As with manufacturing and formulating of pesticides, leaks, spills, and waste disposal associated with treatment plants present a hazard to nontarget organisms. The escape of the pesticides from the treated articles or coating is not known to have contributed significantly to environmental pollution with the possible exception of antifoulant paints where sand blasting prior to repainting has transferred particulate matter to water and muds.

Methods of application whereby pesticides enter the environment

1. Fumigation of enclosed space (chambers, vaults, sealed structures, and space enclosed by impervious tarps) involves the use of toxicants which are volatile, penetrate rapidly, escape rapidly when re-

leased and do not leave objectionable or toxic residue. The quantities used are small compared with other common pesticides. The released gases escape to the atmosphere but quantitatively contribute little to air pollution at today's usage levels. Nontarget organisms are not harmed unless accidentally confined within the enclosed space.

2. Applications of pesticides to soil range from mass treatment at one extreme wherein large volumes of soil are exposed to the pesticide, to seed furrow treatment where the pesticide is confined to a small fraction of the total soil mass. Between these extremes is a wide variety of application methods designed to match the pesticide properties with the vulnerability of the target organism and to do so without damaging the desired plants.

a. Volatile liquids and gases are employed for the control of pests in soil, particularly nematodes. Soil used for seedbeds may be treated for weed, fungus, and insect control. The volatility of methyl bromide requires that a tarp be used to prevent escape of the gas. Less volatile liquid fumigants such as 1,3-dichloropropene-1,3 dichloropropane mixtures or ethylene dibromide are applied by preplant application using chisel or plow applicators after which the toxicant slowly diffuses through the top 12–24 inches of soil depth (if the plow soil is broken up, it is possible to achieve greater depths of penetration) to reach the nematodes. Volatile liquids (1,2-dibromo-3-chloropropane for example) which are less phytotoxic to growing plants can be applied by side dressing using chisel applicators or by irrigation with water containing accurately metered concentrations of the pesticide. Nontarget organisms are also affected throughout the exposed soil mass.

In time, ranging from a few hours to a week or more, the volatile fumigants will disappear from the soil. Effects on soil organisms do not last beyond 1–3 years. Fumigants containing bromine such as methyl bromide or ethylene dibromide leave a bromide residue in soil which can be absorbed by certain plants and become incorporated in feed or food. Growing peanut plants, for example, are unusual in their ability to transfer bromide ion from the soil to the foliage. Only a small fraction of the total enters the nuts. Cows fed peanut hay transfer bromide to the milk. For this reason, bromine containing fumigants are not registered for use in soil where peanuts are to be grown. A more detailed discussion concerning soil fumigants will be presented in a later section on nematocides.

b. The use of water as a transport vehicle to permeate the soil mass and reach subsurface target zones was mentioned above relative to the volatile nematocide 1,2-dibromo-3-chloropropane. Other water-soluble materials can be applied in this way but the practice is very limited. Careful metering and timing is required to prevent overdosage or underdosage. Moreover, soils with high sorptive capacity (organic and clay soils) restrict uniform penetration of the active ingredient throughout the soil mass. Sandy soils with low sorptive capacity are most adaptable because a comparatively even penetration of the dissolved pesticide advances throughout the sand with the carrier water.

c. Mechanical mixing of pesticides into the soil mass to achieve uniform distribution is a specialized method but not widely practiced. Vigorous mixing or rotary tilling is required. Both solubility and volatility are physical properties which aid penetration beyond the original zone of deposition and this results in greater exposure of the soil mass. Insoluble materials move very slowly beyond the point where they are mechanically deposited.

d. Seed furrow treatment is more commonly practiced with insecticides and fungicides. Sprays, dusts, or granules can be incorporated during the planting operation. Proximity of the pesticide to the planted seed can be regulated by positioning the pesticide entry relative to the planter shoe, regulation of rates of addition, and turbulence of the returning treated soil as the seed is deposited and the furrow closed. (Furrow treatment may also be combined with pre-emergent surface treatment to be described below.) Furrow treatment results in high local concentrations limited to a very small fraction of the soil mass. The zone treated is selected to control destructive organisms during germination and early growth stages of the seedling.

e. In highly specialized situations it may be desirable to establish barriers to pesticides to prevent movement of organisms into and through uncontaminated areas. Barriers have been applied to prevent the spread of nematodes to adjoining citrus groves and to prevent the invasion of structures by termites. The applied concentrations are high to afford lasting protection.

3. Granules and pellets afford a means of controlled mechanical application of pesticides without excessive airborne drift beyond the desired area treated. Granules can be applied from ground or aerial equipment. Rates of release of pesticide from the granule can be regulated. In some cases, as with granular application of insecticides, it is desirable to bind the pesticide more tightly to the carrier substance thereby limiting the exposure to direct contact by the insect. In other cases, the granular or pellet composition is designed for release and transference of the contained pesticide to the soil. Subsequent movement depends upon the solubility and sorption of the ingredients. Granules are quite commonly used for the application of insecticides to soil and turf. There is growing use of granular and pellet applica-

tion of herbicides for selective control of deep rooted perennials and woody plants. Granules are used for general vegetation control around industrial sites, tank farms, railroad yards, highways and areas where complete avoidance of vegetation is desired. The use of granules by homeowners for lawn care involves a variety of formulations and combinations of pesticides with plant nutrients. Dense granules and pellets are used as carriers for aquatic herbicides where bottom treatment is desired. In aquatic application, nontarget organisms are exposed to high concentrations in the direct vicinity of the granule. Beyond that, trace amounts of the liberated pesticide would be encountered unless the material is rapidly hydrolyzed and degraded. Granular and pellet application offer the advantages of localizing the toxicant and minimizing direct contact of nontarget organisms.

Granules can be applied where they are directed but subsequent washing rains can cause surface movement of the granules (particularly on light weight carriers) but such incidents are not commonly encountered unless applications have been made to embankments and slopes. Once the pesticide is released from the granule the potential influence on nontarget organisms follows the same principle of movement, degradation and fate regardless of the method of application.

4. The treatment of seed for the control of insects and soil-born pathogens is commonly practiced. The hazard to nontarget organisms is of minor significance once the seeds have been planted. The chief hazard of seed treatment results from accidental consumption by farm animals or poultry. The disposal of surplus treated seed poses a possible hazard if a grower should take the irresponsible measure of selling treated seed in regular channels of trade.

Analytical procedures are sensitive enough to detect this kind of violation even where treated seed grain is blended with untreated grain. The greatest hazard is to livestock and poultry and no environmental hazard from the disposal of such seed is created unless treated grain is dumped where wildlife eat it.

In England, the death in 1960 and 1961 of large numbers of birds such as wood pigeons, pheasants, rooks, and chaffinches and a few mammals including foxes and hares was attributed to cereal seed dressings containing aldrin, dieldrin, and heptachlor. Voluntary reductions in the use of these materials as seed treatment were followed by a considerable reduction in the number of reported bird deaths.

Reforestation is difficult without taking measures to protect seeds and seedlings. Repellants and toxicants have been employed to discourage or kill mice in particular. Seed treatments have not met with uniform success. This practice, however, does not produce significant hazards to wildlife.

5. Dispersion of pesticides via sprays and dusts is the most common method of application. The purpose is to achieve an effective control of the pest with the minimum cost.

a. High volume sprays with larger droplets (or granules as described above) can be better and more accurately directed to the target.

b. Low volume applications involve a small droplet size or extremely small particles which cover well but drift easily in air currents and wind.

c. Invert emulsions and particulate gels have been employed to a limited extent to control particle size and reduce the fine droplets and thereby prevent spray drift of highly potent herbicides.

A wide variety of ground and aerial equipment is available but additional research on effective formulation distribution and placement would substantially help to optimize integrated control procedure. In addition, more effective placement would reduce the quantities of pesticides needed to control the pest.

6. Aerosols, even more than sprays and dusts, are easily carried far beyond the intended target by wind and convection currents. In finely divided form, the pesticide can more easily vaporize and contaminate the atmosphere.

Household aerosols likewise introduce fine droplets into the room air. This requires materials which are safe if inhaled by man. The quantities that escape are comparatively small, however, and have little, if any, effect outside of the immediate area sprayed.

7. The terminal operation associated with application involves clearing of tanks and equipment, disposal of empty containers and occasional disposal of packages which are no longer useful. Washing these materials into streams and sewers can contribute damaging levels of pollution and serious local effects on aquatic organisms. As stated earlier, this is a recognized problem but one not yet solved in a totally satisfactory manner.

In general, the major significance to the environment of pesticides used in and around homes is associated with the disposal of wastes. Used containers, surplus mix, and unwanted concentrates are apt to be rinsed down the drain, dropped in the garbage, or left on the soil or gutter to be washed down the storm sewer. There is no good way to dispose of such wastes. The most practical method today is to place them in the garbage if municipal wastes will be disposed of in a properly operated and located sanitary landfill or an effective incinerator. The pesticide that goes through the sewage treatment plant will end up either in the sludge or in the effluent to some stream, as does that that goes down the storm sewer. In either case, it becomes a part of the pollutants in the aquatic environment. Any surplus diluted mix dumped on the ground has the possibility of being decomposed by soil biota, retained, absorbed in the soil, or washed by surface erosion into storm sewers and thus into the aquatic environment.

Wasteful application of pesticides and the inadequate disposal of wastes continue to be the two most important sources of pollution regardless of the nature of the pest control program.

In each of the steps (manufacturing, formulation, packaging, transportation, distribution), the attitudes of management and operating personnel have varied widely. Some have been diligent in the control of pollution, others have not. When the operator or owner is under heavy economic pressure, there is a temptation to cut corners in order to reduce costs. More effective monitoring of waste effluents will help to identify the offenders.

The applicator is a key factor in the total series of events which governs the magnitude and nature of environmental pollution by pesticides. The occasional user has little knowledge, whereas the commercial applicator or contract sprayer requires considerable knowledge to stay in business. Between those extremes is almost every variation of individual interest, capability, and awareness. Any regulatory process must take into account the importance of this human element in fostering good choices and practices.

THE EFFECTS OF PESTICIDES

The principal classes of pesticides now in use in the United States are listed in this section. For each, a brief statement is presented dealing with the general extent of use, the nature of the effects of pesticides on target organisms, if that is known, and illustrative examples of the impact on nontarget species and groups. It is not possible to summarize all of the available literature in this report but an overview of the nontarget effects is presented.

Many *fungicides* involve heavy metals such as copper, zinc, or mercury, often in an organic molecule, though inorganic salts of copper and zinc are still used in significant quantities (as plant nutrients as well as fungicides). Elemental sulfur accounts for by far the greatest volume of fungicides; combined forms of sulfur are also used. Dithiocarbamates, phthalimides, and quinones are commonly used as fungicides. Wood preservatives, which are at least partly fungicidal in action, include creosote, coal tar, pentachlorophenol, and some chromium compounds.

The wood preservatives are the only fungicides that are widely considered as potentially hazardous to nontarget organisms other than man, but because of the nature of their use, there seem to be few, if any, documented cases of damage. Similarly, the heavy metals must be considered potentially serious environmental pollutants, but their use is rather restricted and so they are apparently adequately diluted without documented hazards, except to the host crop if misused.

Antibiotics are used in the control of certain bacterial diseases of plants. There is always some concern that human exposure may result in sensitization and conceivably beneficial bacteria could be destroyed. No other hazard to nontarget organisms is apparent.

Herbicides, as presently used, do not present serious and widespread hazards to nontarget organisms. With few exceptions, most herbicides have a low order of toxicity to aquatic and terrestrial animals. One of these exceptions is sodium arsenite which is sufficiently toxic and persistent to warrant special precautions and possibly added regulatory consideration. Dinoseb is rapidly degraded but accidental contamination of lakes or streams is hazardous to aquatic organisms.

The most frequently observed nontarget effect caused by herbicides results from spray drift where sensitive plants are damaged. To a smaller extent volatility, movement via soil run-off and carry-over from one crop season to another have caused damage to plants, shrubs or trees. This type of damage can be minimized and controlled by proper formulation and application. Secondary effects from massive kills of aquatic plants can be serious.

2,4-D is known to be readily decomposed by soil microorganisms and this fact has been well established by many investigators. In addition a mechanism for degradation of some related compounds such as 4(2,4-dichlorophenoxy)butyric acid to 2,4-D seems definite.

Several organisms have been isolated from various soils that can utilize 2,4-D as an energy source.

The persistence of 2,4-D in the soil varies with environmental conditions such as the soil type, temperature, and moisture. Field applications for weed control at usual rates have been reported to last from 2 to 14 weeks.

2,4-D breaks down quite rapidly in the soil under normal conditions and is considered to be a transitory herbicide.

Martin's review points out that 2,4-D, MCPA, and 2,4,5-T disappear in the soil and that this is caused chiefly by the activity of soil microorganisms. However, while 2,4,5-T breaks down in soil this occurs at a much slower rate than 2,4-D. There is considerable variation reported in the time for disappearance of 2,4,5-T in soil. Alexander and Aleem found 2,4,5-T present after 205 days as shown by ultraviolet absorption. Whiteside and Alexander found no evidence of a microbial attack on 2,4,5-T as measured by soil respiration. They also point out in this work that there is no microbial effect of 2,4-D or 2,4,5-T when applied at dosages used for weed control. Burger *et al.* reported complete disappearance of 2,4,5-T after 103 days as shown by a bioassay test. Audus found complete detoxification after 270 days. Warren using 8 lbs. of 2,4,5-T in both an amine and ester formulation found that both forms of 2,4,5-T disappeared after 2 weeks in a farmed muck soil. However, in a mineral soil while the amine salt of 2,4,5-T disappeared between 4 and 8 weeks there was evidence of 2,4,5-T activity from the ester formulation after 8 weeks. Woodford and Sagar point out that 2,4,5-T decomposes slower in soil than 2,4-D taking 47.5 days for 80 percent detoxification compared to 16 days for 2,4-D.

There were only a few references found that referred to the isolation of specific soil organisms capable of utilizing 2,4,5-T as an energy source.. Reid, however, also states this was done in his work although no specific organism was named.

2,4,5-T is not usually considered to be a very long lasting herbicide in the soil although it is generally considered to last 2 to 3 times as long as 2,4-D.

There has been a great deal of work done on the effect of the chlorophenoxy acids upon the soil microflora and fauna.

Kratochvil (*203*) pointed out applications as high as 16 lbs. 2,4,5-T per acre had no significant effect on soil microorganisms as measured by the carbon dioxide production of treated soils. Perhaps this can best be summarized by quoting Audus "on the whole the great majority of observations show that with normal practical rates of application there are no adverse effects of the phenoxy herbicides on the total number of microorganisms in the soil." While originally published in 1964, it still seems to be correct. It is of course dangerous to say there can be no harmful effects on all soil organisms but so far there seems to be little practical hazard.

There was little work found on the effect of these herbicides on higher soil organisms. Fox reported that 2,4-D did not affect the numbers of wireworms, springtails, and mites in a grassland soil. Satchell reported that 2,4-D and MCPA had no effect on earthworm populations when used at normal rates.

The rates of movement and decomposition of herbicides in soils is highly dependent upon soil type, temperature and moisture levels. Organic matter and clay retard the rate of leaching and organic matter helps to accelerate the rate of microbial decomposition of herbicides in soil. Some herbicides are persistent enough to carry over from one season to another and adversely affect sensitive crops planted in the second season. Although an economic loss to the grower, this persistence has not resulted in serious ecological disturbance. Trichlorobenzoic acid and picloram are slow to degrade in soils and tend to leach to deeper soil horizons. Specialists in wildlife management have used selective herbicides as a method of habitat improvement. However, the extensive use of herbicides can damage the habitat of wildlife by removing cover species.

The use of herbicides is growing rapidly because of rising costs of mechanical methods of weed and brush control. Continued research on nontarget effects, coupled with close observation of results in the field, will reveal early indications of unexpected ecological change. In this connection, close observation of effects on algae and phytoplankton are particularly important.

Plant growth regulators are closely related to herbicides, and in fact are sometimes the same chemicals used to kill weeds. On the basis of total pounds used annually, plant growth regulators are small in volume compared to herbicides. Brush killers and agents for control of woody plants are considered under the herbicide section. Of the materials presently used, few present a meaningful hazard to nontarget organisms. Of these, sodium arsenite, used to a limited extent as a potato vine killer, and arsenic acid, used as a preharvest desiccant on cotton, have levels of toxicity and persistence sufficient to be of concern. Dinoseb, although readily degraded, is very toxic to fish and care must be exercised to avoid the direct contamination of water.

Paraquat (a postemergence herbicide and dessiccant) and diquat (an aquatic herbicide) have not been considered harmful to fish and wildlife; however, it would be prudent to maintain close surveillance of these compounds in view of their toxicity through skin contact or inhalation.

Nematocides and soil fumigants.—The current national usage of nematocides and soil fumigants, when compared with insecticides or herbicides, is small both in total pounds applied and area treated. The observed effects on nontarget organisms are largely restricted to the direct action on other forms of plant and animal life within the treated soil.

When a nematocide is applied to the soil in sufficient concentration to kill plant-root parasites, other soil microorganisms are either killed or reduced in population. Similarly the invertebrates inhabiting the soil are killed. The actual degree of population reduction depends upon many factors such as soil type, moisture content, temperature, chemical agent and method of application.

After the initial kill or reduction in numbers, certain organisms repopulate the soil quickly (some reach numbers far in excess of those in untreated soils) while others repopulate slowly. With time, which may be a year or longer, the populations tend to re-establish and approach the conditions observed in the untreated soil.

The organisms which oxidize ammonium ion to nitrate and nitrite ions are relatively sensitive to soil fumigants. Their activity may be reduced from several weeks to several months. During this time, ammonium ion accumulates from the decomposing organic fraction of the soil and any added ammonia or ammonium fertilizer will remain as ammonium. If concentrations of ammonium ion are too high, sensitive crops may be injured.

Although transient population imbalances have occurred in treated soils, there is no present indication that soil fumigants and nematocides present a meaningful threat to nontarget organisms which would require special attention beyond continued observation and research in their proper use.

Insecticides and miticides.—There are approximately 400 chemicals registered as insecticides and miticides with USDA–PRD. These toxicants kill insects and mites by interference with essential biological mechanisms but in most cases the exact mode of action is unknown. This applies as well to the arsenic compounds used since the 1800's. The best guess is that arsenic poisoning is due to tissue breakdown and protein precipitation.

DDT affects the nervous system in such a way as to cause death in insects. Mites, however, are relatively tolerant to DDT. Parathion and other organo-phosphorus insecticides are thought to generally inhibit cholinesterase enzymes of the neuromuscular system. A similar mode of action apparently occurs with carbamate insecticides.

There are numerous other insecticides and miticides but generally less is known concerning their mode of action than those mentioned.

Hundreds of reports and summaries now exist describing the effects of insecticides on nontarget species. Dramatic incidents of losses of fish, birds, and other species created concern which has resulted in a large number of descriptions of observed natural field mortalities, a limited number of experimental field observations, and a variety of laboratory experiments. Since insecticides are used in large quantities and have a wide spectrum of biological effects, they have been the center of attention.

Research has not yet completed the urgent task of providing prediction of the significant effects of the uses of these pesticides, but enough is now known to provide reliable examples and suggest some general patterns. Principal attention here is given to the persistent insecticides and miticides, since nontarget effects are more probable because of persistence. Certain vivid cases of effects from short-lived materials are also cited. The following examples are intended to be illustrative, not definitive. Not enough knowledge yet exists to permit quantitative summary of any of the nontarget effects.

a. Phytoplankton

Drifting plant cells in natural waters carry on a large portion of the photosynthesis on the earth's surface. They synthesize most of the earth's organic material, produce most of the oxygen of the atmosphere, and participate in other essential ways in the chemical cycles of the biosphere. Evidence that pesticides may significantly reduce such processes is unusually important. Controlled 4-hour exposure to 1.0 p.p.m. of aldrin, chlordane, DDT, dieldrin, heptachlor, methoxychlor, or toxaphene reduced productivity by 70–94 percent and endrin, lindane or mirex reduced it 28–46 percent. Concentrations of a few parts per billion of DDT have been shown to reduce photosynthesis in laboratory cultures of four varied species of coastal and oceanic phytoplankton, and 100 p.p.b. lowered production 50–90 percent. Swedish research showed that the alga *Chlorella* undergoes great morphological change and decreased photosynthetic rates after 3 days of exposure to 0.3 p.p.b.

These and other observations do not measure total photosynthetic damage, but suggest that such effects may be important and that there is urgent need for further investigation of the effects of pesticides on phytoplankton.

b. Beneficial Insects

Naturally occurring parasitic and predaceous insects control many insect pests. There is no evidence that parasite or predators as a class are more susceptible to insecticides and miticides than are pest species. However, when the plant-feeding species are severely reduced, their parasites and predators are more severely reduced and may be eliminated from the community because they depend upon the plant feeders for their survival. For example, when parathion was applied to a cole crop the number of predaceous and parasitic species were reduced by 95 percent whereas the number of plant-feeding species were reduced by only 8 percent. Following this type of disruption, population outbreaks of the plant feeders occur. Because the parasitic and predaceous species are absent, the plant feeding species increase explosively.

Some parasitic and predaceous species are eliminated in crops because some parasitic and predaceous species are more susceptible to certain pesticides than are plant feeders. In orchards, for example, when DDT was applied for control of apple pests, populations of certain predaceous lady bird beetles which were highly susceptible to DDT were eliminated. Since these beetles were the principal controlling agents for the pest red mite, the mite population subsequently reached outbreak levels and caused severe damage to the apple trees. This particular mite species is not susceptible to DDT and, therefore, was hardly influenced by the chemical which killed the beetle.

Differential susceptibility is also found in the bee pollinators. The honey bee and wild bee pollinators are more susceptible to the insecticide carbaryl than are many other species of insects. Therefore, the continued widespread use of this chemical may have severe effects upon bee pollinators and, in turn, may reduce the pollination of both cultivated and wild plants.

c. *Marine invertebrates*

In the coastal environment, several kinds of organisms are unusually susceptible to chemicals contained in waters flowing from land. Sessile animals, including shellfish and many other benthic species, must tolerate whatever reaches them since they cannot escape. Arthropods such as shrimp, crabs, and most zooplankton are biologically similar to insects and mites and highly sensitive to some of the arthropod poisons. Data are quite inadequate for assessment of all effects, but some cases have been documented.

Worms form an important part of the diet of many aquatic species, but only a single observation on pesticide effects has been seen. Many worms were found dead after treatment of a tidal marsh by 0.2 pounds of DDT per acre.

Molluscs have received considerably more attention, because they are unusually valuable and because they are exceptionally useful as indicator organisms. Oysters and mussels are used in a broad monitoring program for estuaries, and data on pesticide content are available from 175 sites. These soft-bodied animals have notable capacities for biological concentration of pesticides (as well as of heavy metals and virus), and oysters have been noted to accumulate DDT to 70,000 times the concentration in ambient water. DDT, dieldrin, and endrin and other insecticides have been observed in many coastal populations.

Organochlorine insecticides are deleterious to some molluscs at an ambient concentration of 0.1 p.p.b. and they are as a group considered to be about 100 times as toxic as herbicides. A series of tests of the effects of 52 pesticides on the sensitive larval stages of oysters and clams was especially revealing. Survival and growth rates were sometimes reduced, and many of the tested compounds interfered with embryonic development, so that only a portion of the larvae succeeded in metamorphosing to the post-larval stage. This wide-spectrum series showed vividly that each compound must be tested thoroughly, that each species differs in tolerance, and that each stage in the life history may be physiologically different in its response to a chemical.

Crustacea have been observed to receive both direct and indirect damage. Twenty-four hour exposure of blue crabs to 0.5 p.p.m. of DDT killed 50 percent of the crabs, and 0.3–0.4 p.p.b. of heptachlor, endrin or lindane destroyed 50 percent of exposed shrimp in 48 hours. Application of 0.2–0.3 pounds per acre of DDT nearly extirpated populations of small amphipods and isopods in a marshland experiment, and the numbers were reduced for many months. Blue crabs were reduced 10 to 40 percent when exposed to 0.3 pound per acre of DDT, and by 95 to 97 percent when that level was applied several times each year. As with many other sepcies, larval stages are especially sensitive. A study which is now in press demonstrates that 5 p.p.b. of DDT, exposed to crab larvae for 72 hours, caused 100 percent mortality. Baytex and endrin killed 100 percent at 10 p.p.b.; sevin and toxaphene wiped out all larvae at 50 to 100 p.p.b., with lesser toxicity occurring from malathion, phosdrin, and aldrin. Some indications were obtained of the effects of prolonged exposure to sublethal loads, since growth was significantly reduced at 0.75 p.p.b. and 0.50 p.p.b., although not at 0.25 p.p.b.

Indirect toxicity has been seen in heavy mortality among crabs feeding on fish killed by dieldrin, and fiddler crabs feeding on DDT-laden detritus became so uncoordinated as to lose usual defense mechanisms

For a vast array of invertibrate nontarget species, no data or understanding of pesticides are now available.

d. Fish

Lake Trout.—In 1955 when the fish hatchery on Lake George lost 100 percent of nearly 350,000 eggs removed from lake trout, DDT was suspected. Until recently, about 10,000 lbs. of DDT had been distributed for control of gypsy moth and biting flies yearly in the watershed associated with Lake George.

Careful study revealed that DDT completely inhibited reproduction of lake trout in Lake George and several other heavily contaminated lakes in the adjacent Adirondack region. Although the trout eggs contained relatively small amounts of DDT, the fry were killed at the time of final absorption of the yolk sac when they were ready to feed. At 3 p.p.m. of DDT in eggs, few fry survived and at 5 p.p.m., DDT, the mortality was 100 percent.

Again the specific effects of each toxicant upon each species should be emphasized. In the case of lake trout, DDT was the chemical which hindered reproduction. The fish, however, contained larger amounts of DDE, a metabolite of DDT, but this had no detectable influence on the survival of the adult fish. In some birds such as mallard ducks, DDE is the toxicant which had deleterious effects upon reproduction and DDT showed little effect.

Marine.—There are now enough records of kills of fish in open waters and of the result of experimental studies to permit a degree of summarization for fish. Losses of natural populations in which pesticides have been implicated include death of over 1 million fish of 30 species in a Florida marsh during sandfly control efforts with dieldrin, the loss of over 5 million fish in the lower Mississippi River, and many lesser mortalities.

The literature on pesticides and fishes was thoroughly reviewed in a paper in the *Transactions of the American Fisheries Society* in 1968 by D. W. Johnson, and many aspects of the associated problems and research needs were summarized. The acute effects usually involve the central nervous system and result in instability, respiratory difficulty, sluggishness, and, sometimes, death. Chronic exposure may produce massive residue accumulation in fats, damage to liver and kidneys, injured gills, reduced reproduction, slowed response to external stimuli, loss of appetite, restricted growth, lowered resistance to disease and other stress, changed blood composition, the seeking of abnormally warm waters, modified salt metabolism, cholinesterase inactivation, increased oxygen consumption, and other effects.

Any of these may be lethal to the individual and some are obviously threatening to the affected population.

Increased resistance to specific pesticides has been noted in a few fish, but the response of the scientific community to those observations has been mixed. The species of fish are more likely to survive continuing exposure, but the resistant fish apparently contain higher concentrations of the pesticide—and pose an increased threat to consumers, including man.

The availability of the persistent insecticides to nontarget fish is suggested by the report that all 16 commercial fish foods tested for use in a Canadian trout hatchery contained DDT and its metabolites, and some caused 30 to 90 percent mortality among fry and fingerlings. It is now extremely difficult to avoid DDT and its effects.

e. Birds

Much of the significant evidence on the worldwide effects of insecticides have been provided by birds.

Public and scientific concern were alerted by early reports of heavy mortalities among robins which had fed upon earthworms contaminated during insect control programs. This concern was heightened by later evidence on eagles and falcons and their failure to produce young. There are now many important records of field observations linking insecticides to bird injury, and several definitive reports from experimental studies. Only the principal conclusions are summarized here.

There is a syndrome typical of bird poisoning from DDT. Birds can fly poorly or flutter along the ground, then become totally disabled, undergo convulsions and die in a very stiff position with legs extended. Such deaths have been observed at many locations and the evidence links losses to DDT, dieldrin, and other insecticides. Dutch elm disease control reduced robin populations from 185 pairs to zero over a 4-year period in one area. In another area, total bird populations in succeeding years were 31, 68, and 90 percent below previous levels (robins were 69, 70, and 98 percent reduced). Toxaphene has been seen to cause unusual mortalities of fisheating water birds, including white pelicans, egrets, grebes, great blue herons, and gulls. Georgia quail populations declined after treatment of land with heptachlor, and had not recovered after 3 years.

The effects of sublethal exposure of birds to insecticide residues are only partially known. Experimental evidence has shown a wide variety of changes in response to stress, behavior, liver functioning, testicular development, delay in ovulation, metabolism of steroids, and, especially, failure in the deposition of calcium in shells of eggs. The latter is failure of one of the most basic physiological characteristics of birds.

It has been difficult to relate such evidence to occurrences in wild mobile populations, however, and the possible relationships between pesticides and several great population changes has required utilization of several kinds of evidence. The best known cases of population crashes or drastic declines since the mid-forties are among the raptorial hawks and eagles which are carnivores at the ends of food chains. Serious declines have been noted in various regions for the European sparrow hawk, Scottish golden eagle, English kestrel, sharp-shinned hawk, Coopers hawk, osprey and bald eagle. In each case, there is indication that reproductive success has declined, and that the failure is similar to that caused by persistent chlorinated hydrocarbons. For the osprey, Scottish golden eagle and European sparrow hawk a correlation has been observed among frequency of egg breakage, decrease in eggshell weight, subsequent status of breeding populations and exposure to these pesticides. Not all hawks, owls, eagles, and other carnivorous bids show these effects.

The most widely observed species, however, is the peregrine falcon. The population levels in Europe and North America were critically reviewed in 1965 in a conference at the University of Wisconsin, and the proceedings, supplemented by more recent observations, were published in 1969 with Dr. Joseph J. Hickey as editor. Many observations on falcons were provided, including notes on eyries which have been known for centuries but which became vacant in the sudden population losses of the last 20 years. Drastic declines in Finland, Germany, France, Britain, Switzerland, Ireland, Belgium, Sweden, Latvia, and the total loss of nesting peregrines in Eastern United States are described and considered. Several factors may be involved, and they vary from place to place. Reproductive failure is the most probable cause, in the opinion of the conferees, and has involved a failure to lay eggs, decreased number of eggs, egg breakage and eggeating, inability to renest, and decreased viability of the young. In the words of the conference summary, "The ecological case against the chlorinated hydrocarbon insecticides as the pervasive factor in these phenomena is essentially complete." This pattern of evidence is convincing if the portions drawn from different sources point in complementary ways to the same conclusions.

Rigorous experiments to examine the effects of some of the suspected insecticides have recently been established, and results of exceptional interest and significance are now available. The American sparrow hawk, in the same genus as the peregrine falcon, has been bred in captivity and experimentally exposed to a mixture of dieldrin and DDT. One group received low dosage, equal to the residues often found in raptor food items in the field (0.28 p.p.m. dieldrin and 1.4 p.p.m. DDT, net weight); a second high dosage group received a level calculated to be just short of lethal; controls were maintained; observations were continued through the second generation; and partial replication of the entire experiments was achieved. Treated birds showed reduced reproductive success, involving disappearance of eggs, thinner shells, and possible egg eating by parents. The authors, Porter and Wiemeyer of the U.S. Bureau of Sport Fisheries and Wildlife, conclude "The remarkable similarity in pattern of reproductive failure between our experimental hawks and wild raptor populations strongly supports the hypothesis that recent reproductive failure in several raptor populations in the United States and Western Europe were due to common physiological and behavioral responses to intakes of sublethal amounts of persistent chlorinated hydrocarbons." Experimental evidence has also been obtained on mallard ducks, and DDE severely impaired reproductive success, reducing shell thickness and the hatchability of eggs with uncracked shells. DDD was less severe, but also reduced success.

These experiments appear to forge the last link in the chain of evidence that DDT and its derivatives have been the direct and principal cause of widespread and significant reductions in bird populations. The full extent of the damage cannot yet be determined.

f. Mammals

Insecticide damage to mammals has apparently not been as frequent nor as serious as in birds and fish, although the available data are scarce. Occasional mass mortalities have been reported after use of aldrin, endrin and dieldrin.

Field sampling has shown that DDT and its derivatives are present in the fat of many species of wild mammals, including those in areas which are not known to have received pesticidal exposure. A young crabeater seal in the Antarctic contained a small quantity.

The pathways, pharmacology and effects of insecticides in mammals are not well known for wild species, although considerable work has been done on the species useful in laboratory studies (which are treated in more detail in relation to the effects of pesticides on humans). In deer, dieldrin at the rate of 25 p.p.m. reduced reproductive success, with lowered survival and birth weight of fawns. The effects of isomers apparently vary, since o,p′-DDT, which usually makes up 15 to 20% of commercial DDT, acts like estrogen in rats and some birds whereas the more common p,p′-DDT does not.

Only a few wild mammals have been sampled, but it is probable that many or all have now been exposed to the persistent pesticides, that many have accumulated measurable quantities, and that some have been adversely affected.

Piscicides.—In the establishment and maintenance of sport fisheries and management of waterfowl in fresh water impoundments, it is frequently desirable to remove the trash fish. Rotenone is still one of the most important pesticides for this purpose and its dosage can be so controlled that it creates no particular hazard to nontarget organisms except for temporary effects on zooplankton, insects, and other benthos. Antimycin A, an antibiotic synthesized by certain fungi, is a new piscicide which is not toxic to invertebrates or higher forms of life at the low concentrations used, and selectively controls many speices of fish. Since it degrades rapidly, especially in alkaline water, it can be used quite safely in many ways and in types of water where other piscicides may affect water uses.

Toxaphene has been used as a piscicide and is very effective, especially in controlling pest populations of carp and catfish. The manufacturer has discouraged this unregistered use. Toxaphene is destructive to much of the aquatic fauna and is so persistent that residues can prevent a normal fauna for many years. Its actions and persistence is somewhat unpredictable depending in part upon temperatures but also on unknown factors. One lake in Oregon was rendered unproductive of fish for at least 6 years as a result of overdosage of toxaphene. This use in State fishery management programs has long been discouraged by USDI insistence that Federal aid for fish restoration funds cannot be approved or allocated for this unregistered and hazardous use.

Avicides.—A considerable amount of research has been devoted to the search for avicides that are effective in controlling pest species of birds without damaging desirable species. The development of avicides is carried out by personnel who also have a very real responsibility for the protection of desirable species of birds, and their screening tests rule out many candidate chemicals that have unwanted characteristics.

Only a part of the problem is associated with disagreement as to whether or not a particular species, such as pigeons, should be considered a pest. The more important problem is developing the necessary specificity. Much of this problem is solved by formulation and placement of the poison. Thus, there are now chemicals available that can be so placed in feedlots that they are reasonably effective in killing the pest starlings without contaminating the cattle feed. Undoubtedly, some desirable species of birds are also killed, but few of them frequent the feedlots during the wintertime when most treatments are made, so the problem is not acute.

Unforeseen hazards usually come to light during the research and developmental stages of a new compound. For example, one experimental chemical was used on bait placed around a corn field. The following year, wheat was grown on the area, and it was noted that the chemical residue in the soil was phytotoxic to the growing grain.

It must be said that avicides pose a very great hazard to desirable species of birds, but they are not generally used where such problems are serious.

Rodenticides and other mammal biocides.—Certain rodenticides still in use are among the most hazardous pesticides both to man and domestic animals. Sodium arsenite, strychnine, sodium fluoracetate (1080), phosphorous paste, and zinc phosphide are all in this category. Restrictions on labeling and marketing have reduced actual danger to a minimum. In fact, none of these compounds is now generally available and, currently, reports of poisonings of dogs, cats, or children from these materials are very rare.

When these materials are used in the control of predators for rabies control or for plague control, one would expect considerable secondary killing of grazing animals or of scavengers. It is a tribute to the care with which these materials are handled that there seem to have been no massive kills of nontarget animals.

Anticoagulants, red squill, and other rodenticides available for household use perhaps have killed some cats but even this is rare because they are generally of low toxicity to cats. In the case of the anticoagulants, the necessity of repeated exposure has been a significant safety factor.

Repellents.—Several kinds of chemicals are used for repelling insects, birds, and mammals. Insect repellents are applied directly to man and his animals to prevent pests such as mosquitoes and other biting flies from biting and feeding. Repellents may also be applied to the bird roosts to force the birds to move and roost elsewhere. Most of the insect and bird repellents are nontoxic, highly volatile, and degrade rapidly. How these repellents function to repell these pests is not known. Other than causing discomfort to the target organism or nontarget organism which comes within range of the repellent, there is no documented evidence of danger to nontarget organisms.

Various mammal repellents, such as mountain lion dung used against deer, are employed to prevent mammals from entering crop areas. Repellents serve to alter or modify the behavior of mammals as well as other animals without harming the particular species of animal.

ALTERNATIVE PEST CONTROL PRACTICES AND THEIR POTENTIAL DANGER TO NONTARGET ORGANISMS

Whenever pesticide pollution is discussed, the use of alternative pest control practices are suggested as a means of reducing pollution and hazards to nontarget organisms. Most of these alternate controls have been employed against a wide variety of pests during the past 100 years or more. Although research on these alternate pest control practices has increased during the past 10 years, relatively few successes have been achieved and the list of alternate controls remain small. The prime reason for the slow development of these alternate methods is that generally this research requires a great expenditure of time and money. This aspect of pest control deserves greater attention and the research effort should be both encouraged and supported.

Just as there has been some pollution hazard associated with the use of pesticides, there are also dangers associated with some of these alternative controls. Below is a listing of several alternative pest control practices, with comments on their potential danger to nontarget organisms.

Parasites and predators.—In nature, parasites and predators play important roles in the control of many animal species. Although they usually do not provide full control of pest species, parasites and predators are valuable assets in providing partial control of many pests.

Parasites and predators have also provided effective control of individual pest insect and weed species. For example, vedalia beetle imported in 1888 from Australia was highly effective in controlling the Cottony cushion scale insect which caused serious damage to the California citrus crop.

Shortly after 1952 the imported Klamath weed was controlled by an imported weevil (*Chrysolina gemellata*) which originally came from England. This resulted in a restoration of large acreages of pasture in California. A recent instance (1964) of successful biological control involves the use of small wasp parasites (*Aphytis maculicornis* and *Cocophagoides utilis*) against the (*Parlatoria oleae*) olive scale in California. Annual crops such as vegetables and cereals are generally unsuited for control by parasites and predators.

The crop environment which is relatively stable and includes perennial plant types is effective in maintaining parasite and predator populations. Partial control by parasites or predators used in combination with insecticides (integrated control) can be of great value and result in a decrease in the amount of pesticide used for control. This would have obvious benefit to nontarget organisms.

Biological control with parasites and predators is, however, not without danger to nontarget species. Probably the best example of biological control resulting in environmental deterioration is illustrated by the Indian mongoose. The mongoose was introduced into Jamaica in 1870 and subsequently the U.S. island of Puerto Rico for rat control in sugar cane. Within 15 years the presence of mongoose had caused a change in rat species. The ground nesting Norway rat population was reduced by the introduced Indian mongoose. With the removal of its competitor, the tree nesting rat population increased and caused damage to the cane. The mongoose also became a pest itself by preying on poultry and ground nesting bird species and became a reservoir for rabies on the island.

Imported insect parasites and predators may also attack beneficial insect parasites or predators and thus destroy an established pest control program.

In general parasites and predators offer several opportunities for achieving pest control with reduced pollution hazard. If new importations are selected and established with great care there should be few dangers to nontarget organisms.

Pathogens.—The use of pathogens which cause disease in pest insect, and weed populations is the subject of continuing investigation. Under natural conditions viruses, bacteria, fungi, protozoa, and nematodes can be effective control agents. In the United States the introduced Japanese beetle is being controlled to a considerable degree by a bacterium which caused a milky disease in the insect population. Spores of the milky disease bacterium may remain viable in the soil for 20 years, so once an area is infected it remains this way for a long period.

Another bacterium, *bacillus thuringiensis*, can be applied like an insecticide against pest caterpillars which feed on some crops. Using this bacterium, however, is like employing an insecticide because the active agent is a crystalline chemical which is toxic to the caterpillars and other insects. This bacterium does not cause disease epidemics in the pest population like the milky disease.

Several viruses, like the polyhedral virus of the cabbage looper, can give excellent control of pest insects. Although today there is no evidence of danger to humans, they are not being utilized because there are no exact criteria for determining safety to humans and other nontarget species.

Based on the available information about bacteria, viruses, and the other pathogens, there appears to be little danger to nontarget species because of the specificity of most pathogens. Exceptions include *Bacillus thuringiensis* and other pathogens in which elaborated toxins may be involved.

Host resistance to pests.—In nature host resistance is one of the dominant forces limiting the numbers of animal populations. It follows then that host resistance in plants and animals is potentially one of the most effective and safest alternative methods of control.

Host resistance has been used with relative success against several species of plant pathogens. Although plant resistance to insects is known to be common in nature, the method has not been widely exploited. Use of host resistance discourages some economic biologists because of the long period of time needed for selecting and breeding commercial varieties of crops.

Cabbage yellows, for example, has been effectively controlled by breeding resistant cabbages. Hessian fly, a serious pest of wheat, is primarily controlled by host resistance.

Host resistance has several advantages. There is no hazard to either man or nontarget species. The method can be employed jointly with other alternate methods and pesticides without interfering with the effectiveness of the combined control technique.

Environmental manipulations (*cultural control*).—Many pest populations can be reduced or controlled by modifying crop and livestock cultural practices. Some of these techniques are listed below, and it should be noted that generally these cutural controls offer little hazard to nontarget species. At present, some of these have practical limitations which might be overcome through research and additional experience.

1. *Plant spacing.*—Pest damage to crops can sometimes be reduced by altering the spacing of the crop plants. With new crop management techniques which include better control of water and ferti-

lizer needs, crop plants can be grown in more dense stands. In this way the productivity of the plant population per unit time and area may be increased with the net result of a reduction in pest damage per unit plant area.

2. Species diversity.—Monoculture in modern agriculture is a necessity, but with some crops species diversity can be increased by strip cropping or interplanting. Increasing species diversity could result in reducing pest population numbers by increasing the number of enemy species present and improving their efficiency. For example, the white pine weevil causes less damage to white pine grown in mixed stands of trees than when the white pine is in a pure stand.

3. Timing.—Planting time of a crop may be altered such that planting is done after a pest species emerges from its winter resting stage. In this way the pests die before they find suitable crop plants. Hessian fly infestations are partially controlled by planting wheat following the spring emergence of this pest.

4. Crop rotation.—Crop rotation is useful in pest control because it prevents the build-up of pests which live on a crop grown continually in the same area. By following a crop (corn) with a new and unrelated crop (legume), for example, it is possible to avoid large populations of the corn rootworm.

5. Water management.—Draining flooding, and water level control can be used effectively in pest control, but may have drastic ecological effects. With increased control of water use in crop production, additional means are available for pest control. For example, flooding of the soil can drown certain soil pests such as nematodes and soil fungi. Both flooding and draining can destroy some nontarget species in the soil.

6. Fertilizers.—Host plant nutrition affects the longevity and fecundity of a substantial number of pests. By altering the application of nitrogen, potassium, and phosphorus used in crop culture, it is possible to reduce the number of some pests. The longevity and fecundity, for example, of spider mites are adversely affected when their host plants are cultured in soils which are deficient in nitrogen, phosphorus, or potassium.

7. Soil preparation.—Pests, primarily insects, which overwinter or live in the soil during part of their life may be killed by either plowing and/or disking the soil. It has been shown that 98 percent of the pupae of the corn earworm overwintering in the soil is killed by a thorough disking and breaking of the soil.

8. Sanitation.—Various pests can be controlled either by eliminating their alternate hosts or by eliminating the source of infection before it spreads. For example, 90 percent control of the Dutch elm disease is possible in a town or community if a sound sanitation program is carried out. Spraying the elms with an insecticide improves control of the disease from 2 to 5 percent.

Induced sterilization.—Control of the screw-worm fly in Florida and other areas by the mass release of radiation sterilized male flies has been well demonstrated. This sterile male method has many advantages but unfortunately its application for control of many species of pests is somewhat limited. Various chemical sterilants are under investigation for pest control, but these chemicals can be extremely dangerous to nontarget as well as target species. Distribution methods might be found to place the poisons inside protected feeding stations, whereby these chemosterilants would reach only pest species.

Physical methods.—Light can attract various insect moth pests to traps. Light reflections from aluminum foil have been employed to keep aphids from attacking some host plants. Sound may be employed in a similar manner to draw mosquitoes into a trap. Other possibilities of pest control using physical factors need investigation. These appear to have little or no effect on nontarget species.

Genetic manipulation of pest populations.—In the laboratory it has been demonstrated that lethal genes may be released in a pest population to reduce the viability or the numbers of a particular pest population without affecting any other species present in the habitat. Because the method is specific there is no danger to nontarget organisms.

Pheromones and hormones.—Naturally occurring chemicals which are produced by the pest or its host may be employed for pest control.

1. Sex pheromones.—Some insect species may be attracted to traps from up to a mile away by chemical secretions produced by either males or females. Generally these chemicals are quite specific for the insects they attract and, therefore, are not hazardous to other species.

2. Developmental pheromones.—Various chemical messengers which control specific phases of development and growth occur in organisms. These included the juvenile hormone of arthropods which inhibits moulting and development into adult stages. This pheromone has general activity to most arthropods and, therefore, is of potential danger to a wide variety of species. Before these hormones are declared safe for widespread use, information on their spectrum of activity on nontarget organisms should be established, otherwise they may perform as broad spectrum chemical pesticides. The danger to nontarget, beneficial insects and other beneficial arthropod species such as shrimp, crabs, and lobsters could be great.

3. Plant hormones.—Various hormones, which influence growth and development in plants may also influence the susceptibility of these plants to pest attack by insects or plant pathogens. It might be possible to control some of these pests by judicious use of these plant hormones.

COSTS AND VALUE TO SOCIETY

The economic value of pesticides as they relate to crop protection is difficult to quantify, but an assessment of the hidden costs of nontarget effects is even more difficult. A few observations may be gleaned, however, which may be useful approaches to the intangible associated values.

There is indirect evidence of the value that the public places on fishing and hunting. Out of a population of 130 million over 12 years of age in the United States, there were about 50 million hunters and fishermen who spent 650 million recreation-days and approximately 3.9 billion associated recreation dollars in 1960. The Department of Commerce has estimated 33 million hunters and fishermen (23 percent of the population over 12 years of age) in 1965.

The extent of national land resources and the time spent by our people also give indirect evidence of value attached to recreational environment.

	Acres	Visits
National parks (1967)	28, 000, 000	140, 000, 000
National forests (1967)	228, 000, 000	150, 000, 000
State parks (1967)		440, 000, 000
State parks and recreation areas (1965)	40, 000, 000	

Estuaries and the continental shelf represent an area receiving the outflow of water from all land sources and this marine environment supplies 60 percent of the seafood products of the United States with an annual estimated value of $225 million at dockside. Nearly 4 million people hunt and fish in estuarine environments of the country.

Public concern over environmental pollution and threats to certain wildlife species is additional indirect evidence of value although willingness of many to dump beer cans, litter and trash gives evidence that pollution is the "other guy's" problem!

Well-planned changes may have a favorable effect on an ecosystem but if not well conceived may induce a series of complex changes which are subtle and hard to correct. The direct costs of environmental contamination are indicated by the loss of food in which residues exceed established tolerance levels (as in the case of Coho salmon), injury to crops and resulting damage claims, water purification costs and damages resulting from known contamination and spills. These are hard to estimate with any degree of accuracy.

Some relationships of pesticide costs to our economy might be useful as a background for judgments as to whether the added costs of improved materials or practices would be an excessive burden to producers or consumers of food and fiber.

In 1968 total farm production expense in the United States was \$33–\$36 billions and the cost of all pesticides at the consumer level totaled \$1.7 billion of which \$1 billion was spent for direct use in growing food and fiber. Approximately 3 percent of farm operating costs resulted from pesticide materials. (This varies greatly crop by crop.) The cash receipts received by growers for food and fiber were \$44 billion; therefore, the pesticide cost of \$1 billion represented 2.3 percent of total farm product value and 3 percent of farm operating costs. It is estimated therefore that the cost burden of pesticides necessary for the production of food and fiber would range between 1.0 and 1.3 percent of the retail purchases of food and clothing by the consumer. This leads to the conclusion that the economy is able to afford better methods and improved pesticides.

The agricultural productivity of this country can easily be taken for granted in times of plenty. This productivity is a source of great international economic strength for the United States. Incentives are needed to encourage improvement of present pesticide practices and develop new ones which optimize the relation between man and environment.

TRAINING AND LICENSING OF PESTICIDE USERS

There has been a reduction of about 50 percent in the use of DDT in the United States since the peak year of 1958–59. If dramatic additional reductions in the use of this and other pesticides are to be obtained, some system of regulatory restrictions on use will doubtless be necessary. Existing Federal regulations affect use other than by aerial applicators only indirectly (through labeling and through restrictions on residues permissible on produce at marketing).

Two methods of regulating use of potentially harmful substances without completely prohibiting them have been used extensively. One is restricting use to licensed applicators who are expected to use good judgment in their applications; the other is to permit their purchase only on prescription from professionally qualified experts who are expected to use good judgment in instructing the user.

The licensing method has been used by many States in regulating certain types of pest control operators such as structural pest control operators, aerial applicators, or contract agricultural sprayers, etc. This technique has seldom, if ever, been used to restrict use by a farmer or other individual who is making his own application.

The second method is well-established in the restriction of use of dangerous drugs in the treatment of humans and domestic animals. A related method has been used in California for some pesticides where a permit to purchase must be obtained from a County Commissioner of Agriculture.

If either method is to be effective nationally, some method must be found to set nationwide standards for its operations. Since there are already many States employing the licensing mechanism for one or more of several types of applicators, this has the advantage of a core of laws and regulations from which to start. However, the variability of these laws and regulations may make achievement of a national standard more difficult than to start fresh with the second method. Moreover, the licensing of each individual user may be more restrictive of the rights of individual citizens than is necessary.

Either method will require a system of training and testing the licensed user or prescriber and of assuring his compliance with the criteria of use. The basic standards of training, testing, and criteria for use should be set by a national board operating under the aegis of a suitable national professional society such as the American Institute of Biological Sciences, and financed initially by a contract from the Department of Health, Education, and Welfare or other appropriate Federal agency. State or local counterpart boards should be established to carry out the details in their own jurisdictions.

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CHAPTER 4

Effects of Pesticides on Man

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EFFECTS OF PESTICIDES ON MAN

SUMMARY AND CONCLUSIONS

The scope of this report is intended to encompass the present state of knowledge concerning the nature, extent and consequences of human exposure to pesticides. Data relating to exposure of experimental animals have been reviewed only insofar as they contribute to our understanding of phenomena encountered in man or provide knowledge in areas where human data are meager or totally lacking.

No human activity is entirely without risk and this maxim holds for pesticide usage in the human environment just as it does for all other exposure to chemicals. There are formidable inherent difficulties in fully evaluating the risks to human health consequent upon the use of pesticides. In part, these difficulties stem from the complex nature of the problems involved, the fact that many facets of these problems have been recognized only recently, and the general backwardness in this area of research in *man*, as distinct from work in laboratory animals. Above all, one must not lose sight of the large number of human variables—such as age, sex, race, socio-economic status, diet, state of health—all of which can conceivably, or actually do, profoundly affect human response to pesticides. As yet, little is known about the effects of these variables in practice. Finally, one must realize that the components of the total environment of man interact in various subtle ways, so that the long-term effects of low-level exposure to one pesticide are greatly influenced by universal concomitant exposure to other pesticides as well as to chemicals such as those in air, water, food and drugs. While all scientists engaged in this field desire simple clear-cut answers to the questions posed by human exposure to pesticides, the complexity of the human environmental situation seldom allows such answers to be obtained. Attempts to extrapolate from the results of animal experiments to man are also beset with pitfalls. Hence, the greatest care needs to be exercised in drawing conclusions regarding cause-and-effect relationships in human pesticide exposure.

The available evidence concerning such human exposure to pesticides derives from three main sources: planned and controlled administration of pesticides to human subjects; case reports of episodes of accidental or other acute poisoning; and epidemiological studies, which in turn comprise surveys of occupationally-exposed groups (in accordance with a variety of retrospective and prospective approaches), and studies of the general population.

Indices of exposure of human beings to pesticides constitute a vital link in the chain of evidence that must be forged in order to reveal, interpret, and maintain effective surveillance of, pesticide exposures. Hitherto, the view that exposure of the general population was predominantly associated with the presence of pesticide residues in food has been reflected in the efficient monitoring of total diet samples and individual foods, but only sporadic attention to other sources of exposure. It is now evident that much can be learned by monitoring the end-product of human exposure in the form of pesticide levels in body fluids and tissues of people. The information thus obtained is quite distinct from, and at least as valuable as, the data on residues in food; the two types of data complement each other admirably. Provision of information on human levels, in adequately detailed coverage of various groups within the general population is seen as the single most immediate step towards a better understanding and surveillance of total exposure from all sources of pesticides.

Sophistication achieved through the use of modern techniques has made possible the study of absorption, disposition, metabolism and excretion of some pesticides in man. Experience derived from animal studies has provided guidance in directing the appropriate procedures to the investigation of the behavior of pesticides in the human body. To date, the most significant information of this sort relates mainly to two organochlorine pesticide groups, namely DDT and allied compounds as well as the aldrin-dieldrin group. Knowledge of the dynamic aspects of the behavior of these two pesticide groups in the human body is far from complete, but already some important facts have been established. In general, for any given level of pesticide intake, an equilibrium level of pesticide is attained in blood and body fat, despite continuing exposure. The precise concentration at which the plateau is established is directly related to the level of exposure but also to other determining factors. In the case of aldrin-dieldrin, the blood level appears to be a reliable measure of exposure. It appears further, that DDT in blood is directly related to recent exposure, while in contrast DDE in blood is a reflection of long term exposure.

A detailed survey of case reports of incidents involving accidental poisoning by organochlorine pesticides reveals that their general action is to increase the excitability of the nervous system. Some of these compounds also damage the liver. Their capacity to penetrate intact human skin varies from one compound to another; in the case of endrin, for example, percutaneous penetration plays an important part in clinical intoxication. Within the organochlorine group of compounds there is a wide range of potential for acute toxicity: DDT is relatively safe in terms of acute intoxication, while dieldrin and endrin have produced many cases of serious poisoning. Lindane presents a special problem, inasmuch as it has been implicated, largely on the basis of circumstantial evidence, in the causation of hematological disorders. A characteristic of organochlorine poisoning is the difficulty of establishing the correct diagnosis. This is especially true in cases of mild poisoning that result in nonspecific symptoms and signs, since except in the case of dieldrin there are no established criteria for diagnosis on the basis of blood levels. Specific therapeutic measures do not exist.

Inhibition of cholinesterase enzymes by the organophosphate pesticides appears to be the only important manifestation of acute or chronic toxicity produced by this class of compounds. Great variation in acute toxicity from one compound to another characterizes this group, which includes some of the most toxic materials used by man. Cholinesterase inhibition results in a well-defined clinical pattern of intoxication which can be readily diagnosed. Specific therapeutic measures are available and, provided they are pressed with sufficient speed and vigor, are highly effective. Skin penetration by organophosphates may be substantial. In view of the toxic potential of these compounds, protection of workers exposed to them assumes utmost importance. Protective measures should include education, training, proper equipment design, suitable personal protection devices, careful medical surveillance and well-organized facilities ready to treat cases of poisoning with a minimum of delay.

Carbamate pesticides are also cholinesterase inhibitors but, because of rapid in vitro reactivation of the enzyme, measurement of cholinesterase activity is not a reliable guide to exposure. As with organophosphates, the toxic potential of some members of the carbamate group is very great.

Controlled exposure of human volunteers to pesticides under close medical supervision constitutes the most reliable approach to the unequivocal evaluation of long-term effects of low levels of pesticide exposure. The difficulties involved in maintaining such studies have inevitably resulted in very small groups of subjects being exposed for any appreciable length of time. The longest studies on record have lasted less than four years and the results can only reflect the period of study. Consequently, the findings, especially when they are negative, are open to question when taken by themselves. It appears, however, that present levels of exposure to DDT among the general population have not produced any observable adverse effect in controlled studies on volunteers. The same is true of aldrin-dieldrin. These findings acquire greater force when combined with observations on other groups, such as occupationally-exposed persons.

With organophosphate pesticides, the problem of human residues does not arise because these compounds are not stored in body fat. Here the risk is one of acute poisoning. Much accidental poisoning is attributable to public ignorance of the toxicity of these chemicals and neglect of appropriate precautions in their use and storage. In developing countries serious accidents result from storage of pesticides in unlabeled bottles and of food in used pesticide containers. Epidemics of acute poisoning follow spillage of concentrated organophosphates into bulk food or water sources. The hazard to human life is shared by fish and wildlife. Regional pesticide protection teams are suggested as a means of investigating, recording and ultimately preventing accidents of this sort.

Industry has made much progress towards safe handling of pesticides. Nonetheless, a very real occupational hazard exists, and extension of preventive measures should include regular blood testing for evidence of organophosphate exposure. A limit for DDT and other organochlorine pesticides in blood should be established to prevent overexposure.

Pesticide exposure experienced by the population at large is in part the legacy of earlier excessive or injudicious use of persistent pesticides. Residues of these compounds have been, and are still being acquired from all articles of diet and a variety of other environmental sources. This is the major source of public concern. Although a number of persistent pesticides can be identified, attention is centered on DDT, and closely-related compounds, the most ubiquitous and predominant of all pesticide residues in man. The consequences of these prolonged exposures on human health cannot be fully elucidated at present. Evidence from workers who are subject to vastly greater exposure than the public is reassuring but far from complete. Animal experiments clarify certain issues but the results cannot be extrapolated directly to man. On the basis of present knowledge, the only unequivocal consequece of long-term exposure to persistent pesticides, at the levels encountered by the general population, is the acquisition of residues in tissues and body fluids. No reliable study has revealed a causal association between the presence of these residues and human disease.

Despite such reassurance, realization of the paucity of our knowledge in this area flows from increasingly sophisticated studies on human residues of DDT and related compounds. There appears to be marked geographical stratification of DDT residues in our population, the average levels in the cooler isotherms being one-half of those in the warmer climates. None of these observations apply to residues of dieldrin. Such findings cast serious doubt on accepted beliefs that food is the predominant source of DDT residues and that the entire general population has reached equilibrium as regards acquisition of such residues.

Reopening these questions emphasizes the inadequacy of present monitoring of exposure by relying mainly on analysis of food. This aspect was stressed above. It also renders more urgent the need to contain and eventually greatly reduce the extent of human and animal contamination by pesticide residues. Existing knowledge confirms the feasibility of inducing active withdrawal of pesticide residues from the human body but further research to achieve a practical means of attaining this goal is needed.

A survey of the reported effects of pesticides on laboratory animals has furnished information on factors and experimental conditions that could not easily be reproduced in human studies. For example, the influence of diet on pesticide toxicity, and particularly lack of dietary protein, has revealed substantial increases in acute toxicity of some pesticides. In this, as in some other sections of our report reference is made to the capacity of organochlorine pesticides to bring about a great increase in the activity of liver enzymes responsible for the metabolism of foreign compounds. This phenomenon of enzyme induction has been extensively studied in animals and is discussed in detail in the report of the Panel on Interactions. Comparable enzyme induction in the human liver is brought about by many drugs and also by DDT. It is a sad comment on the dearth of knowledge of human physiology to point out that the threshold dose of DDT for induction of metabolizing enzymes in human liver is unknown.

Special sections of the report deal with the possible effects of pesticides in bringing about heritable alterations in the genetic material (mutagenesis), effects on reproduction, including malformations in the fetus or newborn infant (teratogenesis) and increasing the incidence of various forms of cancer (carcinogenesis). The data available relate only to experimental animals or to lower forms of life. At the present time we do not know whether or not such results are applicable to man. While there is no evidence to indicate that pesticides presently in use actually cause carcinogenic or teratogenic effects in man, nevertheless, the fact that some pesticides cause these effects in experimental mammals indicates cause for concern and careful evaluation. It is prudent to minimize human exposure to substances producing these adverse effects in mammals while additional investigations are undertaken to assess the potential of such suspect pesticides for causing adverse effects in man. There is a need to develop standard protocols for safety evaluation that are sufficiently flexible to permit an individual approach to the particular and often unique problems presented by each pesticide. Assurance of safety to man demands special techniques, not only for extrapolation of animal data to man, but also for evaluation of controlled human expsure. Much effort will be required to attain these objectives. Research in these areas should be expanded and imbued with a greater sense of urgency than that manifested before.

The Panel on Interactions has provided a valuable analysis of the manner in which pesticides can interact with one another, and with drugs and other environmental agents, in exercising effects on man and animals. Once again one is struck by the complexity and importance of these interrelationships and by the extent of our ignorance of effects on man.

To sum up, the field of pesticide toxicology exemplifies the absurdity of a situation in which 200 million Americans are undergoing lifelong exposure, yet our knowledge of what is happening to them is at best fragmentary and for the most part indirect and inferential. While there is little ground for forebodings of disaster, there is even less for complacency. The proper study of mankind is man. It is to this study that we should address ourselves without delay.

NEEDS

Improvement in the present situation requires:

I. Organization of resources for effective continuing action.
II. Improved registration and review practices.
III. Clarification and strengthening of laws and regulations
IV. Action to improve the health of the public.
V. Initiate programs to evaluate and provide a system of graded actions for existing contamination or contamination which cannot be controlled at the source.
VI. Research and investigations.
VII. Industrial cooperation.

I. *Organization of resources*

In view of the current organization of the Departments involved in the control of pesticides, and inter-Departmental relationships, there is a need for a single organizational unit that might be called a pesticide board at the level of the Department of HEW to make the final risk-benefit judgments for the guidance of the Secretary. The board through a central staff and peripheral operational units is intended to fulfill the following functions:

1. Organization and legislation.—Supervise the regulatory, registrative and review activities either directly or through other operating units; serve as the principal planning and budgetary organization for the Secretary in the pesticide field; coordinate and promote an overall

Federal pesticide control program with the other Departments having a major interest in pesticides, i.e., the Department of Agriculture, Department of the Interior, and the Office of Science and Technology.

The board should consider the most effective way or organizing existing resources currently designated as pesticide activities, specifically the activities of the Bureaus of Science and Medicine of the Food and Drug Administration. It should also consider pesticide resources currently related to the National Institutes of Health, the Health Services and Mental Health Administration, other Administrations of Consumer Protection and Environmental Health Service, and the regulatory, review, laboratory and investigative resources in the Food and Drug Administration.

2. Service.—Establish investigative teams and monitoring systems; collect data on pesticide contamination of man and his environment; set up educational and preventive programs throughout the operational units.

The board should initiate plans for a coordinated surveillance system based upon giving first priority to measurements of human exposure or measurements which can be quantitatively related to human exposure. This involves supplementation of programs which monitor food for regulatory purposes. Preferably, human tissues and body fluids should be the basic indices. The system should provide information for the board on its risk-benefit judgments, and data to give perspective to the regulatory programs related to specific media (food, air, water) or formulations (pesticide registration). All information from Federal, State and foreign sources regarding any aspect of pesticide usage, pesticide related human morbidity and mortality experience, ecological impact, etc., should be put into retrievable form. This information should be made available to governmental agencies, the news media, industry and the public-at-large. A principal use of this data bank would be the identification of areas of ignorance. It could also inform the public of progress being made toward safe use of pesticides.

The board could develop a plan to focus existing training and education activities and to determine deficiencies and develop resources to fill the most significant gaps.

Education of the public should begin at the earliest possible age and continue throughout the school and college career. How to live with toxic chemicals should be as ingrained into the public mind as the precautions necessary to cross a highway. Parents, teachers, chemists and everyone else in any way involved should be encouraged to participate in the development of general awareness and understanding of the principles of safe use of pesticides and other toxic chemicals in and out of the home.

3. Information.—Obtain, analyze and publish relevant information on the effects of pesticides on human health and environmental quality. Expert interpretation of the significance of the findings is an essential part of this function.

The board could also immediately consider methods of improving and consolidating the existing periodicals related to pesticides, the Pesticide Monitoring Journal of the Federal Committee on Pesticide Control and the Health Aspects of Pesticides into a periodical providing information on all the health aspects of pesticides. Expert critical interpretation of the significance of this information has hitherto been lacking, and should be provided.

4. Research.—Establish priorities for research related to exposure and effects, methods of sampling and analysis, and epidemiology; establish investigative teams, monitoring systems, and evaluative organizations.

II. Registration and review in order to reduce present and future exposures

The registration of any pesticide, persistent or otherwise, should be subject to periodic review and reapproval by the board and in no event should a pesticide registration be effective for a period longer than two years.

A. The present residue tolerances and registration of persistent pesticides should be reviewed immediately to restrict the various uses presently authorized by current registrations only to those that are essential for protection of public health or essential for high priority production of food and fiber where no effective non-persistent pesticide or alternative control methods are available. The use experience of each registered pesticide should be reviewed every 2 years. Provision should be made to withdraw registration wherever use experience is unfavorable for health reasons or ecological reasons. Input from health agencies, agricultural agencies, universities, conservation groups, etc. should be sought.

B. Registrations granted for new pesticides should initially be granted only on a provisional basis with restricted usage while ecological and biomedical data on persistence, biomagnification, and adverse side effects on man's health or his environment are being developed.

C. New pesticides characterized as persistent (as defined in report of *contamination* in Chapter 2), or likely to undergo significant biomagnification, or potentially threatening man's health or the quality of his environment should not be granted a provisional registration unless deemed necessary for protection of public health and/or essential production of food and fiber.

III. Legislative and interdepartmental agreements

The Federal Insecticide, Fungicide and Rodenticide Act should be modified so that any use of a pesticide can only be registered: 1. with the approval of the Secretary of the Department of Health, Education, and Welfare; 2. after consideration of possible consequences and environmental contamination.

As an interim step, the interdepartmental agreement (Federal Register, May 1, 1964) should be clarified so that procedure of label review for USDA by HEW and USDI shifts the burden of proof from the Federal agencies to industry (the applicant for registration). Specifically, section 2(d) of the Agreement should be revised to delete the requirement that the Federal agency which objects to a label registration must support its objection by "appropriate scientific evidence." Both industry and government should be expected to supply sufficient evidence to enable the proposed HEW pesticide board to reach a decision.

IV. Action to improve protection of the health of the public

A. Strict limitation of permitted uses of DDT to those that are essential for public health purposes.

B. No extension in present limited uses of aldrin, dieldrin, heptachlor and heptachlor epoxide, BHC and lindane.

C. Discourage home use of persistent pesticides.

D. Prohibit introduction of pesticides of intermediate or high toxicity into the home.

E. Exercise more ingenuity in the design and development of home baits to reduce the hazard of human and animal intoxication.

F. Monitor effluent from pesticide manufacturing, formulating and distribution plants.

G. More efficient application of pesticides by means of improved equipment and better-trained personnel (see other sub-group recommendations).

V. Provide for a system of evaluation and graded actions for contamination unsusceptible to control at the source

Many persistent pesticides in the environment will continue to contaminate foods and other media even though their use is discontinued in a given part of the nation or world. The quantities in the oceans will continue to build up for many years and the biological magnification processes will focus on some specific food chains that reach man. This type of contamination is not susceptible to control at the source. Further, it is not caused by the food producer or food processor. Therefore, rather than exerting control through a single numerical tolerance level at which the food is removed from the market, it is recommended that a series of graded levels and actions be established for pesticides, leading to the decisive action of removal from the market. These might include:

- Grade 1—Levels of concentration requiring only general surveillance.
- Grade 2—Levels of concentration requiring measurements relatable to exposure in humans.
- Grade 3—Levels of concentration requiring assessment of human intake and exposure and initiation of actions to reduce concentration through variations in harvesting, processing, and distribution techniques.
- Grade 4—Levels of concentration requiring removal from the market.

Such a series of graded actions would probably be unique for each group of pesticides and perhaps for each active substance, and in some cases for specific combinations as they exist as contaminants. Considerations for evaluations, while related generally to concentration levels, must basically relate to exposure of a susceptible group of the population. The consumption of a food and its replaceability in the diet must be given adequate consideration as the several gradings are established. Likewise, the state of the evaluatory art and science, and the capability of the sampling and analysis systems must be weighted in the grading of the pesticide with regard to both the concentration in a given food product and the health implications of human exposure to the pesticide.

VI. Research and investigations

A. Urgent research needs:

i. To achieve more detailed understanding of the mechanisms of absorption, distribution, metabolism, storage and elimination of pesticides in man.

ii. To define the impact of other environmental chemicals on the pharmacokinetics of pesticides on man.

iii. To elucidate the effects, if any, of pesticide exposure on human exposure to drugs and other environmental chemicals.

iv. To delineate the influence of age, sex, various nutritional and disease states and climatic conditions on these aspects of man's response to pesticides.

v. Areas of special concern: these relate to studies on human volunteers aimed at investigating under conditions of controlled exposure such aspects as sensitization; behavioral effects; significance of lowering of blood cholinesterase; reduction of body burden of pesticides by dietary and therapeutic measures.

vi. Development of improved methods for detection of hepatic, renal and CNS effects of pesticides in man.

vii. Investigations in animals. It is imperative that work in animals be directed more closely to a practical objective: the need to maximize our understanding and control of the effects of pesticides in man. Accordingly, attention should be given to more detailed study of the following aspects of pesticides:

a. Effects of exposure on hemopoiesis, heme synthesis and other biosynthetic processes.

b. Carcinogenesis, mutagenesis and teratogenesis brought about by pesticides, including the development, evaluation and interpretation of testing procedures.

c. Interaction among pesticide effects and between pesticides and other chemicals found in the environment.

B. Investigative needs—pesticide protection teams: The pesticide protection team is envisaged as being a area-wide three-man multidisciplinary team with investigationary, recording, and health and safety promotional responsibilities. The investigationary role will call for the exploration, investigation and documentation of episodes of pesticide poisoning and environmental contamination. Prevalence levels of pesticide residues obtained by collecting meaningful samples from man and wildlife will be monitored and surveillance of cholinesterase and other biological indices of exposure in the exposed worker promoted. In addition, the uses of pesticides will be documented and reviewed. By example and education a more informed and safer community atmosphere will be engendered. It is proposed that a sanitarian, a representative from the county agricultural agency, and a representative of fish and wildlife form this three-man team, reflecting the participation of health, agriculture and wildlife ecology. Their functions can be described under the following categories: investigation and reporting of episodes, surveillance, monitoring and education.

a. Investigation and reporting of episodes.—The team would:

i. Set up a reporting system of human pesticide poisonings and significant fish or wildlife perils due to accidental misuse of pesticides.

ii. With the assistance of appropriate local agencies, carry out a field or site visit at the scene of the incident, documenting the circumstances and causes of the poisoning, collecting the appropriate samples for chemical analysis, and taking the necessary steps for the prevention of similar incidents.

iii. Compile and submit all episodic data to the environmental toxicology organization at the regional level.

b. Surveillance:

i. The regular clinical surveillance of the occupationally exposed worker should be promoted. Clinical intoxication should be prevented by routine cholinesterase testing. Workers should be withdrawn from further exposure when blood studies are indicative of hazardous absorption. The program should be extended to cover agricultural laborers, part-time employees, migrants, crop dusters and swampers, formulators and manufacturers.

ii. Pesticide practice both in urban and rural communities would be reviewed. From time to time appropriate soil and water samples would be collected to ascertain that chemicals are not being misused or inappropriately used. Obvious environmental sources of pesticides such as smoke and waste effluents from manufacturing and formulating plants would be monitored on a regular basis.

VII. Industrial cooperation

A. Higher standards should be set in respect to:

i. Design of containers: lips, closures, proof against mishandling, corrosion.

ii. Labeling of containers including simplification of names on domestic pesticide containers.

iii. Disposal of containers: Systems should be considered which will insure the return and/or proper disposal of containers. These should include a plan for disposal in the application for registration, and notification to public health authorities of the nature of the pesticide, and the precautions that must be taken to avoid hazards to the public health during the disposal processes.

iv. Limitation of "blunderbuss" pesticide or pesticide-fertilizer products.

PREFACE

The sections of this report were developed in several ways, varying from individual authorship and review by the Subcommittee to sections developed by the Subcommittee as a whole. The sections on *Interpretative Pitfalls* were written by the Subcommittee.

Pharmacokinetics of Organochlorine Insecticides was written by Dr. J. Robinson, Tunstall Laboratory of Shell Research Limited, London. Dr. Wayland J. Hayes was the principal author of *Controlled Human Exposures. Epidemiology of Pesticides* was largely the work of Dr. John E. Davies and Dr. Thomas H. Milby. *Clinical Case Reports* was authored jointly by Drs. Dudley P. Miller, Griffith E. Quinby, and Thomas H. Milby. Dr. George B. Hutchison was the author of *An Appraisal of Hazards to Man from Long-Term Exposure to Pesticides.*

Cutaneous Aspects was prepared by Dr. H. I. Maibach. *Behavioral Effects of Pesticides* was developed by Dr. Griffith E. Quinby. Dr. William F. Durham wrote the section on *Experimental Animals*. Dr. J. H. Wills prepared the document on *Preventive and Therapeutic Measures* from which the final version was developed.

The other sections were initially drafted by one member of the Subcommittee but generally reflect the thinking of all the members. It should be noted that Dr. John E. Davies, while officially listed as a special assistant, served as a full member of the subcommittee.

The Commission recognized that some aspects of the biological effects of pesticides required special study. *Carcinogenesis*, *Mutagenesis*, *Teratogenesis*, and *Interaction* were designated as Panels by the Commision in order to reflect the special interest attached to these subjects and to facilitate in-depth evaluations of the available scientific evidence by additional experts in biomedical sciences. The subcommittee on Human Effects had liaison association with the Panels, but their organization, evaluations, summaries, and conclusions were determined by the Panel membership, not the Subcommittee on Human Effects.

INTRODUCTION

We accept that today, as throughout man's history, safety is a relative term. In any of his activities, whether awake or asleep, man cannot achieve absolute safety. While the risks to health that abound in the home, in the street and at work are accepted as inevitable and are limited as far as possible, the hazards to health that stem from environmental exposure to chemical agents are usually beyond the capacity of the individual to control. By their very nature—such as chronicity or subtlety of effects produced—the risks deriving from this source constitute an altogether different dimension from all others (except for radiation) in their threat to human safety. Pesticide exposure is but one sector of environmental chemical hazard, yet its problems typify the complexities of the chemical sophistication of our society.

Our concern here is with the impact of pesticide exposure in all its facets on the broad perspectives of environmental health in man. In this context our definition of health necesarily encompasses more than the absence of disease. Included in our view of health is the feeling of well-being and capacity for happiness that derives from a suitable environment: suitable in the sense that it makes possible the enjoyment of nature and her bountiful provision of flora and fauna. From this standpoint therefore any factor or activity that detracts from the variety of the environment, that reduces its capacity to contribute to health, is fundamentally undesirable. This broad generalization must, however, be tempered by the practical realities and priorities of human existence on the earth.

The need to provide food and other crops and to prevent or eradicate insect-borne disease constitute problems which many countries must necessarily regard as outweighing in importance the potential or even actual hazards to health involved in the use of pesticides. Hence we must recognize at the outset that protection of human health involves a system of priorities which are necessarily different from place to place. A good example from another field, that of food additives, is provided by the use of hydrogen peroxide to preserve milk. This practice would not be tolerated in countries where the customary methods of milk distribution are available. Yet the Joint FAO/WHO Expert Committee on Food Additives recognized that in some areas of the world safe milk would not be available at all unless a preservative could be added, and hydrogen peroxide appears to be effective for this purpose.

Much of this section of the report is taken up with discussion of toxicological complexities, epidemiological uncertainties and a frank acknowledgement of the vast areas of ignorance in our understanding of the effects of pesticides on man. Some of the reasons for this state of affairs will be discussed below. It is appropriate here to consider two aspects: What degree of proof is necessary or desirable for a decision to be reached that a health hazard exists; and who has the burden of proof in this regard?

On the question of degree of proof, a course must be steered between the two extremes usually encountered, namely the tendency to jump to hasty conclusions not warranted by the available data and the insistence on complete and irrefutable evidence before action can be taken. The problems have to be considered from the standpoint of a reasonable man, fully cognizant of the present state of the art in the sciences that constitute the basis of safety evaluation and apprised of all the facts at present available on the pesticide problem. Actions based on conclusions that go beyond the available information are only warranted if there are reasonable grounds for the belief that the risks of present practices outweigh the benefits. The risk verus benefit equation thus enters into this, as into all other judgments on safety-in-use of chemicals.

The burden of proof that the benefit derived from the use of a pesticide exceeds the risk is usually considered to lie with the manufacturer. Regulatory authorities, both national and international, already insist on substantial evidence of safety and efficacy which takes into consideration the nature and amounts of residues on various commodities.

In recent years the manufacturers have also had to pay increasing attention to ecological implications of the use of their products. As times goes on, however, and the realization grows of the wide dimensions of the problems presented by pesticide usage, it is becoming abundantly clear that no manufacturer nor group of manufacturers can be expected to investigate and deal with the repercussions of pesticides in our society. They may be rightfully expected to contribute to the cost of surveillance and research in the broad areas of epidemiology, but the organization and execution of this all-important task is the responsibility of the community. It is the province of governments and international authorities to concern themselves with investigations and subsequent decisions regarding proof of safety to human health.

The judgment arrived at by reasonable and informed opinion involves an intelligent appraisal of possibilities, an assessment of uncertainties with a measured degree of confidence. In weighing potential health risks against potential benefits it must never be forgotten that even the most far-seeing view may be proved erroneous by unexpected new scientific developments or by an altered attribution of those risks considered to be of utmost importance. An instance may be cited in the area of nonnutritive sweeteners. Earlier safety evaluations took into account softening of stools as the likely risk presented by high intake of cyclamates. Now one source of concern is the possibility of carcinogenesis brought about by these products or materials derived from them. Thus safety evaluation is an edifice whose construction is never completed; nor does it remain functional without periodic reconstruction. Strangely enough, both regulatory agencies and the public view as loss of face the frank recognition that many earlier decisions on safety must inevitably be proved wrong as scientific knowledge grows. There is nothing absolute about such decisions. All that we have a right to expect at the time they are made is that they should be the products of scientific competence and experience, mature judgment and full possession of all existing data.

The decisions that now confront us with regard to DDT exemplify the issues at stake in the judgments to be made concerning pesticides. Firstly, there are the quantitative aspects of the amounts accumulated in man's environment and their very slow rate of diminution, even when all further cumulation ceases. What is true outside man is equally representative of internal storage of DDT and DDE in his tissues. In attempting to relate this situation to any possible health hazard to man we must not fail to take into account the implications of chemicals that may be used as alternatives to DDT. Such substitutes, and the problems associated with them, have been referred to in earlier sections of this report. We must also recognize that not every "effect" brought about by DDT in man is necessarily detrimental. For example, the capacity of DDT to stimulate liver processing enzymes, a phenomenon discussed in detail below, has been put to therapeutic use in a case of familial unconjugated non-hemolytic jaundice. The patient was rendered anicteric and remained so 7 months after cessation of treatment with DDT. The possibility always exists that the pesticide of today may find application as the drug of tomorrow. Such active agents also offer opportunities for "depesticidation," that is the safe withdrawal of pesticide residues from the body.

Naturally, these lines of thought invite the objection that a treatment beneficial in the context of a clinical situation is not necessarily a boon to healthy individuals and even less so to patients with other illnesses, to the very young, to pregnant mothers and their unborn infants, and to the aged and infirm. Exposures that exercise a negligible, hence acceptable, effect in normal healthy individuals have to be assessed quite separately in their impact on these special groups of the population. For control of hazard one needs to take into account the most susceptible members of the community and to delineate selectively the possible toxic effects which each human condition may involve.

INTERPRETATIVE PITFALLS

This section seeks to explain some of the reasons why simple answers are not readily forthcoming to most of the questions concerning human exposure to pesticides. By having in mind the complexities and difficulties of the situation we are better able to attain the objective of reasonable assessment set out above.

Toxicological complexities

a. General.—Toxicology is directed towards the evaluation of safety, basing its conclusions on studies of chemical composition and reactivity, physical properties, degradative or metabolic transformations undergone by the materials involved and biological effects of potentially injurious agents. Such biological effects are assessed by means of observations of alteration of structure, function and response in living systems.

Just as a physician relies on a grounding in basic sciences to achieve clinical understanding, so—to an even greater extent—does the toxicologist for the variety of systems and organisms with which he has to deal. Effective consideration and elucidation of the complex relationships that exist between dose of pesticide, route and duration of exposure, time of observation and target organs affected necessitates a multifaceted approach. In DDT we have a striking instance of acute effects directed towards the central nervous system, while long-term exposures involve the liver as a primary organ of attack. For each compound, in each experimental or epidemiological situation, only a comprehensive review of the problems is likely to provide the correct perspective. Anything less than an all-embracing approach can lead to serious distortions of fact and errors of interpretation. A wide range of scientific disciplines needs to be brought to bear on each problem so that their contributions may be integrated into a well-rounded overall assessment of hazard, from which flows the necessary balanced judgment on safety-in-use.

While it is desirable that those involved in safety evaluation have as broad a scientific background as possible, so many types of expertise are involved that toxicology inevitably becomes the effort of a multidisciplinary team. Even with the participation of several individuals, each skilled in his own specialty, the pace of scientific advance is such that an increasing timelag has developed in the application to toxicology of new knowledge generated in the basic sciences.

Other factors also militate against progress in toxicology commensurate with that occurring in other branches of science: the small number of adequately-trained toxicologists, the limited facilities available for training such experts, the lack of understanding and hence of interest of the academic community in the problems of toxicology. Most serious of all is the handicap to progress presented by toxicology's heritage from its past. The procedures for evaluation of safety developed by the early pioneers represented attempts to grapple with pressing problems, using mainly intuition based upon the body of knowledge available at that period. The effluxion of time has hardened these approaches into concrete routes for achieving acceptance of a product by the regulatory authorities. Departures from established routines involve risk and expense. Accordingly there exists a built-in incentive encouraging repetive performance of old procedures and discouraging innovation, particularly the exploration of radically-new routes to safety evaluation.

Just how serious the consequences of such attitudes are will become clear from consideration of the many areas of ignorance to which this report will call attention. Realization that such gaps in knowledge exist is the first step towards remedying the situation; but further steps will necessarily be slow in coming as long as the application of new knowledge is dependent on workers in the basic sciences. Here toxicology must fend for itself. Hence there must be provided within toxicological laboratories multidisciplinary teams of critical size that will have as their primary task the development of new approaches to the many unsolved problems in the area of pesticide effects on the human body.

b. Special features of pesticides.—The large heterogeneous group of compounds covered by this report comprises distinct and well-defined classes whose properties—physical, chemical and biological—bear little relationship to one another. Their pattern of use is a complex and changing one, with the result that human exposure assumes highly intricate and often unpredictable characteristics, depending on place, time and other circumstances to be discussed below.

Although pesticides lend themselves to consideration in groups, it is essential to realize that each product, even more than each compound, is an individual problem peculiar to itself. The ideal of selectivity, that is specificity of action limited to one type of insect or plant, is the theoretical objective to be aimed at in developing the "perfect" pesticide. We must recognize frankly that this goal is unattainable. Just as no drug can be expected to restrict its effect to the desired receptor, so with any biologically-active compound one must anticipate a wide range of actions on a broad variety of living systems. We have learned to live with this inescapable fact in the case of drugs. We must accept it as fundamental in our consideration of pesticides. Thus from the standpoint of human health, selection of one pesticide rather than another involves a balance of risks which are not qualitatively comparable; in other words, there are as many differences as similarities in metabolic pathways, pharmacodynamics and biological effects between groups of pesticides and within each group. It is this diversity that militates against sweeping generalizations and dogmatic conclusions.

Of all the particular features of pesticides that make their consideration a special problem in toxicology, the quality of persistence has special significance for man and animals, as it has for all other parts of the environment. Prolonged retention in the body is by no means an exclusive feature of pesticides. Components derived from food or other sources are also stored in tissues, by virtue of their lipophilic character coupled with resistance to metabolic degradation, or their tendency to be taken up by the reticuloendothelial system. Storage may serve the purpose of conservation, for instance of iron, or of segregation, as with heavy metals or lipofuscin pigments, rendering materials potentially dangerous to the cell as innocuous as possible while they remain in storage. While pesticides are not unique in being stored, nevertheless they probably represent in many instances the highest levels of foreign material present in adipose tissue and perhaps in liver. The very fact that certain pesticides have been studied fairly intensively from this point of view makes possible a consideration of the phenomenon of persistence, whereas little useful of the sort could be said about other classes of exogenous compounds finding their way into storage sites in the body.

c. Agent and environmental variables.—Some of the complexities of the pesticide problem arise from the fact that these are technical-grade materials often containing by-products and other impurities whose nature and proportions may vary from one source to another, may vary (in countries where adequate control is not exercised) from one batch to another from the same manufacturer and may change under conditions of storage. The accompanying impurities may themselves play an important part in the biological effects of a pesticide, for instance in human sensitization to a product. Moreover, the impurities may interact with one another and with other compounds present in a pesticide formulation. This raises the question of the many formulating agents that are used and the effects of storage on the toxicity of the vehicles.

Changes in the compounds involved in the application of a pesticide also occur under the influence of environmental factors such as temperature, sunlight, plant metabolism and degradation in the soil, depending on terrain, pH, humidity and intensity of ultraviolet irradiation. Translocation through the environment occurs by a variety of mechanisms discussed earlier in this report. Together with food-chain magnification, the changes undergone by the pesticide in the course of translocation tend to complicate the picture further.

d. Limitations of studies under experimental conditions.—No account of toxicological complexities would be complete without referring, however briefly, to the animals in which biological effects are studied. While some progress has been made in recent years in the provision of healthier and more uniform stocks of laboratory animals, variations in the response to a chemical will always continue to be manifest as a result of species, strain, sex, age and individual differences in susceptibility.

One objective of toxicological investigation is to delineate at least some of these differences and to ascertain in the most sensitive animal species the maximum level of exposure that elicits no adverse effect. Using this level as a basis for extrapolation, an "acceptable daily intake" for man is arrived at by applying an arbitrary "safety factor."

Some of the weaknesses of this approach are readily apparent. Practical considerations must always limit the number of species, strains, etc. of animals investigated. The criteria used and tests applied to ascertain that no adverse effect has occurred are often the best available, but methodology has not yet been developed specifically for this purpose, to a degree permitting measured confidence that adverse effects have not been overlooked. Much more work is needed in this area, both in the direction of sensitive and specific methods of detecting changes and in an attempt to distinguish more clearly between those effects that represent physiological adaptation and those which constitute pathological change.

The study of pharmacodynamics and metabolic disposition of a pesticide in one or more animal species is an important part of the investigation of that compound. In conjunction with the other animal studies just referred to, delineation of metabolic pathways helps to validate the exposure of human volunteers to the compound. For however extensive and thorough the animal experiments may have been, extrapolation of the results to man is still fraught with uncertainty, which may be reduced substantially by investigating the compound in man.

The considerations, limitations and safeguards that enter into the use of human volunteers for research of this sort are now well recognized and widely accepted. Limited though such studies must necessarily be, both in duration and level of exposure, they do yield invaluable information, obtained under controlled conditions, that no other approach can provide.

Epidemiological complexities

Man, the definitive host in the epidemiological interreaction with his environment, has been found to possess many variables over and above his overt pesticide exposure potential, each of which donates subtle additions to the magnitude of his body burden. Thus, beside his occupation, the amount of physical protection that he uses through wearing of clothing and masks, his age, his diet, his race, his socioeconomic state, his home, the drugs he is taking; all are factors which make a significant contribution to the amount of pesticide he absorbs.

Human experience represents a continuum, both of intensity and duration of exposure. The greater part of the American public fall at one end of this continuum, ingesting and absorbing very small amounts of pesticides as residues in foods. In addition, an appreciable number of this general population group receive further exposure to pesticides applied in their homes and gardens for pest control purposes. The relative contribution of these two sources, diet and casual home use, to overall general population exposure has not been fully studied. It is probably safe to say, however, that both sources contribute significantly and each must be considered in any detailed assessment of pesticide-health effect relationships.

Workers who manufacture, formulate, apply, or otherwise come into contact with pesticides in the course of their occupation may be generally considered as moderately exposed to these poisons and thought of as occupying the mid-range of our pesticide exposure continuum.

On the extreme exposure end of the continuum are those unfortunate individuals who, through accident or design, contact and assimilate large quantities of poison and case a result suffer acute, overwhelming and sometimes fatal intoxication.

As the term continuum implies, there is no clear demarcation between these exposure groups. However, experience has shown that each can be identified with sufficient precision to allow investigators to make meaningful observations of pesticide assimilation, accumulation and elimination; study morbidity—mortality patterns as they relate to pesticide exposure; and to a limited extent, determine dose-response relationships in human subjects.

The epidemiological approach.—Since all persons have some degree of pesticide exposure, the epidemiological comparison of exposed and nonexposed is impossible. Comparison of health experiences have to be made between persons or populations with relative degrees of pesticide exposure. Insofar as acute poisoning is concerned, this is a reasonably simple procedure. The effect is an acute toxicological phenomenon and the contributions of person, place, and time to this, together with information provided from toxicological data, make it possible for causal factors to be identified and methods of prevention readily determined.

Acute or overwhelming exposure to one or more pesticides followed by overt illness has in many instances been documented in the medical literature. Knowledge obtained from reports of homicides, suicides, and accidental ingestions of pesticides has provided most of the scanty information available from which dose-response estimations can be developed. These events, along with overwhelming occupational exposures, have provided clinicians with opportunities to describe the acute effects of many of the pesticides. Some indication of age, sex, and race difference in susceptibility have been observed and reported. In addition, acute poisoning events have provided sound evidence that some pesticides may produce permanent damage to health.

More complex, however, is the investigation of the consequences of the less intense but more sustained exposure. Here man is exposed either by occupation or by incidental exposure such as is experienced by the population at large. The contribution of dose and the duration of exposure is very important. In these two situations two populations, the occupationally exposed and the general population have been studied and the epidemiological strategy has been somewhat different with respect to those two different population groups.

Studies of the occupationally exposed.—Investigation of the occupationally exposed has two goals.

1. To assess the health consequences of occupational exposure and to explore the hazards of the industry, to identify areas where the skills of industrial hygiene and occupational health can be applied to protect the health of workers.

2. To investigate the health experiences of the exposed worker with the purpose of using information of a disease occurrence for the purpose of extrapolation to the health effects in the general population. This utilizes the dose effect principle of toxicology and presumes that, if any health hazard which is the result of pesticide exposure is observed, then it will be most frequently and intensively found in the pesticide worker whose exposure is more intensive and prolonged.

Work-related exposure to pesticides among manufacturers, formulators, applicators, field workers and others in related occupations can best be described as infinitely varied. Not only is there great variation in intensity and duration of exposure but in type and combination of pesticides involved.

Scientific studies of pesticide exposed workers have generally been of two types. The first, and most common, of these has been the study of acute pesticide induced illnesses related to single large or multiple small exposures. This kind of event and the information to be gained from its understanding will be discussed in the following section. A second type of study to which occupationally exposed groups lend themselves is the comparative epidemiological investigation.

This form of study may be used to compare virtually any number or combination of health related observations made among pesticide exposed workers to like observations made among workers not so exposed. If properly designed and controlled, the comparative epidemiological investigation is potentially the most sensitive measure of variations in morbidity and mortality experienced by groups exposed to any agent suspected of affecting human health. Once variations are identified, hypotheses regarding causation can be formulated and tested. Unfortunately, there has been a disappointing paucity of comparative epidemiological studies among pesticide exposed workers. Moreover, several studies which have been reported are of limited value because of faulty design. As a result, although a very large number of workers have been significantly exposed to a multitude of different pesticides during the last decade and a half, surprisingly little is understood of the long-term health consequences involved. An additional, perhaps more significant loss may have accrued from our failure to adequately document the presence or absence of adverse health effects related to long-term occupational exposure to pesticides. It has been suggested that illnesses related to long-term exposure to pesticides are likely to be detectable first in workers who are heavily exposed in comparison to the general population. If so, our lack of information in this area may, in effect, deny us the knowledge necessary to predict or anticipate specific pesticide related health effects destined to become apparent in the general population only after many years of low level exposure.

Theoretically, four permutations of cause and effect variables exist in studies such as these. Thus, all pesticides and all diseases can be investigated, or single pesticide and all diseases, or all pesticides and a single disease, or a single pesticide and a single disease can be studied. Methodologic and interpretative pitfalls exist in all four of these designs.

All pesticides—all diseases.—The null hypothesis here is that occupational exposure to pesticide has no adverse effect on health experience. Retrospective or prospective studies have reviewed mortality or morbidity experience in the pesticide worker, comparing them with these experiences in a population not so exposed. If the null hypothesis is accepted, no greater incidence of mortality or morbidity patterns would be observed than was found in the general population controls stratified by age, race and sex. The pitfalls herein are essentially concerned with the type of population comparisons. It is easy to obtain age, race and sex specific mortality data from National or State general population groups. In almost all studies where mortality experience of a single occupational group is compared with a general population group, rates tend to be lower in the specific occupational group than the population at large. This is to be expected since the availability of being employed or occupied is a priori, a characteristic of health. Comparisons then would be more meaningful with another occupational group using as close a match for physical and social characteristics of the two occupations as possible. Thus, it is preferable to compare pesticide exposed workers with another occupational group rather than the general population. Samples of such comparison groups are policemen, postal workers, and the like.

Single pesticides—all diseases.—Several studies have reviewed morbidity data from manufacturing or formulating plants wherein exposure has been predominately to one insecticide. Studies such as these have two interpretative pitfalls which tend to limit the potential information which can be extrapolated to the general population. These are: (1) the number of persons studied is inevitably small and even if expanded by person-years computations the numbers are usually still small, (2) they provide information on survivors only, since mortality and morbidity experiences of dropouts is usually unavailable. Recently in the United States, an attempt has been made to overcome the problem of small numbers of occupationally exposed workers by supporting community pesticide studies in 16 States and combining the clinical data of occupationally exposed persons in all these areas. In addition to recording incidences of established diseases, by subjecting the group to a uniform battery of multiphasic screening tests, information on pre-clinical disease is also being accrued. Thus, a sizeable cohort of workers heavily exposed to pesticides has been made available for an in-depth evaluation. The inclusion of multiphasic screening techniques has highlighted the problem of interlaboratory variability, making interpretation of results difficult. Differences may be observed, which are still within the range of the accepted norms. Even if scrupulous care is used to define the criteria of exposure, clearly differentiating them from the control population not only by occupational history but by their biologic indicies of exposure as well, the blood chemistry differences may be adaptive or compensatory rather than injurious. In addition, an exhaustive search for dropouts is essential, and any significant failure to do this would seriously jeopardize the interpretation of results. No better example of the consequences of this omission can be given than was the case with workers occupationally exposed to asbestos. Mesothelioma, a well known complication of pulmonary asbestosis was not recognized for several years, because dropouts from this industry were not traced.

All pesticides—single disease.—As with other studies of the occupationally exposed, the relative risk due to pesticide exposure must be very high and the incidence of the disease very high for an effect to be recognized in studies where the number of participants is small.

Single pesticide—single disease.—Investigation in this situation usually follows earlier epidemiologic or toxicologic leads. The implication of pesticides in a causal role is established following either toxicologic or hypersensitivity principles or with planned human or animal exposure.

Prospective versus retrospective studies.—The problems of these types of studies are chiefly methodological and logistic. The limitations of prospective studies are their cost, the need for a large number of participants in the cohorts, and the time or period of observation. The turnover of personnel in the pesticide industry is high, which in itself presents a serious logistic problem. Retrospective studies are easier since they are less costly, and the required information is provided in a shorter period of time. However, by and large, only mortality data are available and difficulties due to a small relative risk might occur, making it possible that the disease effect might be missed. Meaningful interpretation can only be deduced from such studies if there is reasonable evidence that a large proportion of the dropouts have been accounted for. Retrospective studies usualy precede the more costly prospective studies, and if a specific disease entity is identified in the former an association only (with no causal connotation) is identified. The evidence for causality is usually obtained from a prospective study and is strengthened by the demonstration of dose and temporal associations. Lastly, studies such as these, even if showing illnesses which appear to be causally associated with the occupational exposure to pesticides, provide little information as to which is the specific offending pesticide.

General population studies.—The conventional approach of assessing the health effects of pesticides in the general population has been to find the dose relationship in the occupational exposed and to extrapolate from this to the general population. This was one of the approaches in radiation studies but was found to be of questionable validity since the diseases were different with different dose exposures. Thus with the continuum of radiation exposure, marrow aplasia, thyroid malignancy, and leukemia was observed. An alternative approach that was used was to do studies of the background radiation level itself. The same approach is possible with pesticides using the human pesticide residue as the marker; but here, as with radiation, work must first be done on the pesticide level itself. Fat and blood have been the tissues of greatest interest; DDT and its metabolic derivatives the principal pesticide studied. The reasons for these choices are clear. Blood and fat are relatively easy to obtain by venepuncture or biopsy at surgery or post mortem examination. Chemical analysis of these tissues has proved to be informative both on an individual and general population basis. DDT has received a major share of interest because of its widespread use, the ease by which it can be identified, and its almost invariable presence in detectable amounts in human adipose tissue (fat).

As discussed elsewere in this report, a few other pesticides of the organochlorine family can often be found in adipose tissue samples obtained from the general population sources, but these pesticides have received much less interest and attention than DDT. Pesticides of the organophosphate type are not found in the adipose tissue or blood of the general population.

It is surprising and somewhat disturbing to note that despite the tens and perhaps hundreds of thousands of adipose tissue analyses done in this country and elsewhere, there are still important gaps in our knowledge of the storage, metabolism, and significance of DDT in human tissues. For example, there appears to be no convincing evidence available at the present time to clearly indicate whether tissue storage of DDT and its metabolites is increasing, decreasing, or remaining constant.

In the face of this mosaic of host agent environment interreaction, it is not surprising that the question as to whether long-term exposure to pesticides is harmful or not remains unanswered. It will require the combined contribution of physiology, toxicology, pathology, chemistry, and epidemiology to answer the question with any degree of totality.

Case reports.—In the past 25 years, thousands of clinical case reports of human illness apparently caused by pesticides have appeared in the medical literature of dozens of countries. An effort has been made here to organize representative examples of this vast body of information in a coherent way. Findings have been critically assessed, and those which appear scientifically tenable have been arranged according to the human organ system which seemed most affected, or according to a disease entity or syndrome when that was more appropriate.

Case reports in which pesticides are implicated typically describe clinical observations of an individual patient (occasionally more than one), pathological findings, etiology or causal relationships, and a regimen of treatment and its outcome.

Whenever information permits, a consideration of dose-response relationships is included here. However, in the very nature of the phenomenological approach, there is often little or no opportunity for clear documentation of extent of exposure. The patient does not seek medical attention until his illness is overtly manifested; by then, it is often impossible to recall or reconstruct how much of a given pesticide was ingested, inhaled, or absorbed. There is a danger of *post hoc, ergo propter hoc* reasoning in this approach.

Errors of omission, however, may be even more serious than errors of commission. The reporting physician, for example, may have failed to study some physiologic system, such as the central or peripheral nervous system, when in fact there was an effect. Once errors of either commission or omission find their way into print, they are often exceedingly difficult to correct.

Studies of groups occupationally exposed.—At least two basic aims should be borne in mind, when designing studies of individuals exposed to pesticides in the course of their employment. Ideally, such studies should generate information on exposure—effect relationships which can be extrapolated from the more heavily exposed occupational group to the less heavily exposed general population. But in addition to this pure research purpose, an applied research purpose is always implicit: Such studies should provide the basis for development of preventive medical programs to preserve the health of the exposed workers. All reasonable efforts must be made to protect workers from harmful exposures at all times. However, should low level exposures be unavoidable, or should massive accidental exposures occur, opportunities to make meaningful observations of health consequences should not be overlooked.

Extrapolations from occupationally exposed populations to the general population always require qualification. The most significant difference between the two groups is that the general population includes large numbers of very young, very old, ill and disabled, while such persons are found rarely or not at all among the occupationally exposed. These individuals are often least resistant to the effects of toxic agents; it is therefore apparent that dose-response relationships based on studies of formulators or other occupational groups may be extrapolated to the general population only with great caution.

Analytical complexities

An appreciation of some of the more important analytic and interpretive problems peculiar to the organochlorines is essential for perspective in this area.

A recently-discovered source of error in analysis of DDT and related materials by gas chromatography is the presence of polychlorinated biphenyls (PCB). Since 1929, polychlorinated biphenyl liquids, resins, or solids have been spread widely in our environment in oils, hydraulic fluids, adhesives, plastics, building materials, fuels, fire retardants, heat transfer agents, electrical equipment, paper, and many other industries. The presence of PCB has caused serious analytical errors in the nonspecific gas chromatographic analysis of the chlorinated hydrocarbons (Jensen, 1966; Richardson, 1969; Reynolds, 1969). It is clearly necessary to confirm qualitatively the determination of DDT residues and not to be confused by gas chromatographic peaks that overlap DDT but represent totally different materials (Schechter, 1968). Such false peaks have been reported in gas chromatograms from some wildlife samples, along with organochlorine pesticides (Roburn, 1965). Cod liver oil from Norway gave rise to gas chromatographic peaks in the region expected for DDT, DDE, and TDE, but paper chromatography indicated the presence of halogenated compounds which were not known pesticides at all (Eidelman, 1963). In samples for the Nature Conservancy in Britain, compounds were detected interfering with detection of p,p′DDT and p,p′TDE (Harrison, 1966). Since 1966, the presence of PCB has also been noted in the British (Holmes, *et al.* 1967; Holden and Marsden, 1967; Robinson, undated reference cited in Richardson, 1969), Dutch (Koeman, *et al.* 1969), and North American (Risebrough, *et al.* 1968) environments.

Polychlorobiphenyls have been identified in fish, wildlife, and the environment as cited above, but no attempt appears to have been made to look for them in human tissues or excreta. Pooled samples of human milk from Colorado Springs and eight individual samples from Berkeley, Calif., were positive for PCB (Risebrough, 1969; personal communication). Past reports of levels of chlorinated hydrocarbon pesticides might thus have been too high as a result of the presence of PCB. A further source of error might be introduced by storage of specimens in plastic containers (Quinby, 1966).

Analyses of environmental samples must be supported by qualitative and quantitative confirmation of the results. Too many papers have been published that incorporate no such quality controls; nor have potential sources of error been considered. Especially when the results are reported as positive near the lower limits of detection of the gas chromatographic method (which is often the case), the results may be absolutely misleading. The mass of material in question is then also insufficient for qualitative and quantitative corroboration of the results by other techniques of analysis.

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Complexities of Terminology

A large body of information has been published regarding the health consequences of human exposure to pesticides. Review of these publications reveals that the scientific terminology is often imprecise and nonuniform from one publication to another. Particularly troublesome and confusing are adjectives commonly used to qualify the terms "exposure" and "effect". Terms frequently used to describe exposure are acute and chronic, high level and low level, and short term and long term. Among the terms that have been used to describe effect are acute and chronic, transient and permanent, and immediate and delayed. While it is not within the scope of this report to propose a standardized exposure-effect nomenclature, the absence of uniformity among authors in this respect must be emphasized.

The terms "acute toxicity" and "chronic toxicity" also deserve comment. Acute toxicity usually implies overwhelming intoxication with overt illness or death. Often only one pesticide is involved and the diagnosis is relatively clear-cut. The term chronic toxicity is, as a rule, more difficult to interpret. The author usually refers to illness resulting from long term, relatively low-level exposure to pesticides. The data concerning intensity and duration of exposure are often unclear or incomplete.

Contributing to these interpretative pitfalls is the concept of the acute LD_{50}. This term refers to the quantity of pesticide which, when administered in a single dose, is lethal to 50 percent of a group of test animals. The LD_{50} is usually expressed as milligrams of pesticide per kilogram of animal body weight (mg./kg.). The species, strain, age, and sex of the experimental animal, route of administration, concentration of test material, and vehicle in which active agent is administered must be specified. Even the most carefully established LD_{50} data cannot be extrapolated to man except in a general fashion. Thus, while it is true that pesticides which are highly toxic to the experimental animal are usually quite poisonous to man, dose-response relationships are usually very different.

This is only one of the uncertainties involved in extrapolating animal data to man. In recent years, a great deal of attention has been devoted to this question. The conclusion that emerges is that only studies in man can provide definitive answers to the questions posed by human exposure to pesticides and other chemicals. Results of experimental studies in animals are at best a guide, providing what are often valuable clues to the nature of the effects that should be looked for in human studies. The fact that every man, woman, and child in this Nation is exposed to pesticides in one form or another demands that answers be forthcoming that are known to be valid for man.

The available information on the effects of pesticides in man usually relates to episodes of acute and often single massive exposure in individuals not previously conditioned or adapted to such exposure. Much uncertainty attends any effort to use this type of information as a basis for conclusions regarding exposures that are long term, low level, and continuous or oft-repeated. These are the types of exposure experienced by the general population. Nor does information relating to healthy adult volunteers or to occupationally-exposed workers necessarily apply without serious modification to the large number of other categories of the general population: the very young, the old, the debilitated, in pregnancy, and in disease states.

It is in this context that one must consider the interpretation of "no effect." In animal studies whose results are used for regulatory purposes, the term "no effect" refers to absence of adverse effects. The line of demarcation is often not clearly drawn between physiological adaptation, which is presumably a beneficial response to exposure, and pathological change, which constitutes a breakdown of the body's defense mechanisms. As increasingly sensitive tests are applied in animal studies, so more and more effects are observed that cannot, in the present state of knowledge, be assigned with any certainty to either the physiological or pathological category. For a variety of reasons, such tests have not yet been applied in man, and hence the absence of effect as judged by conventional criteria is not an adequate expression of what might be found by application of methods combining greater specificity and sensitivity for detection of pesticide effects.

In the course of considering manifestations of pesticide exposure, a clear distinction should be drawn between measures of exposure and measures of effect. For example, pesticide levels in blood and tissues are indicators of exposure. In themselves, they do not constitute evidence of an effect, however, striking the level may be. In the case of plasma or erythrocyate cholinesterase level, moderate or extreme lowering—though not invariably correlated closely with the clinical state of the subject—is a sufficient danger signal to be regarded with due concern and appropriate remedial action taken. A variety of changes in clinical biochemical parameters have been reported in individuals exposed to pesticides. While some of these may ultimately be shown to be useful measures of exposure, or reflect physiological changes within the body, we have no evidence that any one of them is an indicator of toxic effect.

Questions of terminology enter into our discussion of many issues concerning pesticides. It must be made clear that the term "persistent" has no application in physiological terms. The fact that a compound remains in the environment for a sufficient length of time to be carried over from season to season or from one crop to the next, bears no direct relationship to its capacity to be stored in, and mobilized from, human adipose tissue.

The context in which the term "health" is used is important. As mentioned in the introduction, health encompasses, in addition to human health and well-being, the healthy existence of beneficial flora and fauna in man's environment. Public health refers to questions connected with specific human diseases. Environmental health is that aspect of human health dependent upon, or conditioned by, man's environment. Finally, the expression "environmental quality" (as discussed in various sections of the Commission's report), is distinctly different from health, despite the dependence of health on environmental quality. Environmental quality has significance in a scientific sense, an administrative sense, and both nationally and internationally. There is no simple direct relationship between human health and environmental quality, and caution must be exercised in order to avoid drawing conclusions based on the assumption that such a relationship exists.

Need for perspective

Some general explanatory comments are needed on the subject of irreversible long-term effects of pesticides. From the standpoint of the toxicologist all such effects are equally undesirable, whatever their nature, or whatever the organ system involved. In other words, to the expert in the field, the hazard of development of peripheral neuropathy or aplastic anemia must be taken just as seriously as risks of carcinogenesis, teratogenesis, or mutagenesis. There is a tendency on the part of the lay public, the news media, and even among some scientists who specialize in the areas of cancer, or birth defects, or genetics, to regard these particular hazards as transcending all others and, therefore, claiming most immediate and urgent emphasis.

Whatever one's point of view, there is an inescapable factor in this situation that is often overlooked. This is the difficulty of establishing with any certainty carcinogenic, teratogenic, or mutagenic potential for most compounds that are only weakly active in one or other or all these directions. A powerful carcinogen such as diethylnitrosamine or aflatoxin (or unequivocal mutagens such as some alkylating agents), presents no difficulty in clearcut characterization of effects in animals and in assessment of the carcinogenic hazard presented to man. But pesticides do not pose such straightforward problems. Methods for detecting weak carcinogenic potency are still inadequate to provide unequivocal results and hence leave room for differences in interpretation. Methods for evaluating weak mutagenic potential in mammalian systems are even more primitive, and the results even more difficult to apply for practical purposes. Even in the area of teratogenesis it can be said that extrapolation of animal results to man is fraught with great uncertainty.

Such being the present parlous state of the art, one need hardly be surprised that differences of opinion should exist among scientists knowledgeable in the field of toxicology. Even more diverse are the views expressed regarding regulatory action to be taken on the basis of experimental findings. It may be argued that any well-founded suspicion against a compound should suffice to have its use restricted or totally prohibited. Prudence, it would seem, demands no less. At the risk of appearing to be imprudent, one must point to the inescapable fact that there is no chemical present anywhere in our environment, and especially any natural component of our diet, that is incapable of yielding alarming results in some biological system at high enough levels of exposure. Consequently, the circumstances of intended use of the material, and particularly the likely levels of exposure, are of fundamental importance in assessing safety.

Viewed in this light, the attitude that it is impossible to define a safe level for man of a weak carcinogen, or a weak mutagen, is a retrogressive approach to a problem that will not be solved by prohibiting from use every compound possessing an index of suspicion, however low. The argument is indisputable that, despite our state of ignorance, or even because of it, we ought not to add to the existing burden of carcinogens, or mutagens, in our environment. But a generalization of this sort must be tempered by quantitative considerations. If the additional hazard represented by a compound is in all probability trivial, then we have a responsibility to weigh those benefits conferred by use of the compound against the hazard that its presence in the environment represents.

The key issue posed by this line of reasoning is the determination of what constitutes a trivial addition to the existing burden in the environment. The sad fact we have to face is the almost total absence of information on the existing burden of carcinogens, mutagens and teratogens *naturally* present in food, drink, water, air, etc. Not the smallest effort has been made to assess this background, using the available tests, so as to achieve some perspective in judging the results of these same tests with new compounds. Man's exposure over countless generations to a wide variety of naturally occuring toxicants, and the known effects of such exposure (in terms of morbidity and mortality) should be used as a firm baseline from which to judge additional hazards. For example, we know that man and animals constantly ingest in food and drink a variety of agents that are liver toxins and carcinogens. One might therefore anticipate that liver cancer would be a major problem throughout the world. In fact it is so rare that those localized areas of high incidence are almost certainly attributable to special circumstances peculiar to the regions involved. In the United States the incidence of liver cancer is low and there is no evidence that it is rising. Thus, despite lifelong exposure of the entire population to numerous weak—and even potent—natural carcinogens in food and drink, the actual hazard in practice is, as far as we can tell, very small. It is background information of this sort that is urgently needed before assessment of hazard to man from new compounds can be based on realistic judgment.

PHARMACOKINETICS

Routes of Entry

Pesticides may gain entrance to the body through the intestine subsequent to ingestion; through the lungs as a result of inhalation of airborne pesticide-laden dusts, vapors and aerosols; by penetration through the intact skin; and (rarely) by absorption directly into the bloodstream through the broken skin.

The relative importance of these pathways varies, to a large extent, according to the population group under consideration. In the general population, ingestion of residues remaining on foods is probably the major route by which pesticides enter the body. Inhalation may be a factor, particularly in lower-income households, where "bug-bombs" are used in the effort to control cockroaches and other pests. It is unlikely that percutaneous absorption is an important route of entry among members of the general population, at least insofar as acute intoxication is concerned. A possible exception may exist when unrestricted sales of highly toxic pesticides, such as parathion, are permitted for household and garden use. Home poisoning cases are usually accidental, usually involve children, and ingestion is overwhelmingly the most common route of entry.

Occupationally exposed persons, on the other hand, are at risk of hazardous absorption of pesticides primarily through inhalation of dust or droplets generated during pesticide manufacture, mixing, or application. Under some conditions of work—most notably agricultural employment—skin contamination followed by percutaneous absorption may constitute a significant pathway.

The pharmacokinetics of organochlorine insecticides

The dynamics of an organochlorine insecticide in vertebrates may be discussed phenomenologically, that is, the experimental results *per se* may be considered without any preconceptions. Such a treatment of the results may take various forms but it is convenient to discuss them with respect to four different relationships, relationships which are relevant to various aspects of the interpretation of the epidemiological data. These relationships between the experimental variables are: Those between the concentrations of an insecticide in the body tissues and the intensity of the exposure; those between the duration of exposure and the concentrations in the tissues; those between the concentration in the blood and the concentrations in other tissues; and, those between the concentrations in tissues and the time since exposure ceased. Subsequently, we may consider the implications of these relationships, the theories or models that may be proposed as explanations for these empirical relationships and, finally, the inferences that may be made in relation to the epidemiology of these compounds in man.

I. Review of experimental studies of the dynamics of organochlorine insecticides.—(i) Relationship between the intensity of exposure and the concentrations in tissues: The entry of an organochlorine insecticide into the body may occur either by ingestion, inhalation, or percutaneous absorption. Ingestion is the only route of entry which has been systematically studied in relation to the residues arising in body tissues. Fortunately, this also appears to be the major route of entry of the insecticides into the bodies of most members of the general population.

DDT: Laug and Fitzhugh in one of the earliest studies of the store of DDT in tissues concluded that the concentration of DDT in fatty tissue of rats was correlated with the level of DDT in the diet in the range 100–800 p.p.m. (*1*) Hayes (*2*) reviewed the evidence up to about 1958 and concluded that "when other factors are kept constant, the peak storage of DDT in each tissue varies directly with the daily dose." That this conclusion was warranted by the data he reviewed is shown by figures 1 and 2 which are reproduced, for convenience, from Hayes' review. The results obtained by Durham *et al.* (*3*) in a study of the storage of DDT in the body fat of Rhesus monkeys also show that there is an obvious correlation between the concentration of DDT in body fat and that in the diet of the monkeys (see fig. 3). In a study in which DDT was given to human volunteers Hayes *et al.* (*4*) concluded that the storage of DDT in man was proportional to the dosage. Using the results of this trial, and those of a second trial (*5*), Durham *et al.* (*6*) derived a graphical relationship between dosage and storage of DDT in man. The results used by Durham have been used to construct figure 4. The relationship between the mean (arithmetic) concentration of DDT in subcutaneous fat and the daily dose of DDT has been calculated:

$$\log_{10} C = 0.0142 + \underset{(\pm 0.105)}{0.6539} \log_{10} (10^2 \times \text{daily dose of DDT, mg.})$$

where C is the concentration of DDT in fat, p.p.m.; where the value ±0.105 in parentheses is the standard error of the slope of the regression line. The relationship is not exact as arithmetic means have been used in deriving an equation involving the logarithms of the variables; strictly the geometric means should be used but the published results do not allow these to be calculated.

Dieldrin: It has been shown that the concentrations of HEOD in the tissues of rats and dogs (*7*) are related to the daily intake (see fig. 5). Gannon *et al.* (*8*) concluded that in steers, hogs, lambs, cows, and sheep the amount of dieldrin stored in the body fat appeared to be proportional to the rate of intake. An examination of their results (see fig. 6) indicates that they have been rather cautious in drawing such a tentative conclusion. In man, it has been shown that the concentrations of HEOD in whole blood and adipose tissue were correlated with the daily intake of that compound (*9, 10*), and explicit relationships have been derived (*10*); these are:

$$\text{Amount of HEOD ingested per day } (\mu g) = \begin{cases} \text{(concentration of HEOD in blood)} \\ \quad 0.000086 \\ \text{(concentration of HEOD in fat)/} \\ \quad 0.0185 \end{cases}$$

Conclusion: All the available experimental evidence indicates that the concentrations of DDT and HEOD in the tissues of experimental animals and of man are related to the dietary intake.

(ii) Relationship between the duration of the exposure and the concentrations in tissues—DDT: Hayes (*2*) concluded that the degree of storage of DDT in the adipose tissue of rats varied directly with the number of doses until a peak or plateau was reached, but that the true-dosage relationships were much less well-understood for other organs. Results consistent with this concept of an approach to an upper limit of storage, characteristic of the particular daily intake, have been obtained by Ortega *et al.* (*11*) in the case of the rat (see fig. 7), and by Durham *et al.* (*3*) in the Rhesus monkey (see fig. 8). Hayes *et al.* (*4*) concluded that human males achieve storage equilibrium for DDT in about a year (see fig. 9), but that further observation was necessary to establish this conclusion firmly. Hayes (*2*) commented that after the plateau concentration had been achieved there was a tendency for the concentration of DDT in the body fat of rats to decline. There is an apparent tendency for a similar decline in the concentration of DDT in the body fat of the Rhesus monkey (see fig. 8), but Durham *et al.* (*3*) suggested that this may be an artifact caused by random variation and the loss of some animals from their respective groups.

Dieldrin: The concentrations of HEOD in the tissues of rats and dogs approach an upper limit of storage characteristic of the daily intake (*7*), and this phenomenon is illustrated in figure 10, based upon the results obtained by Deichmann *et al.* (*12*) in a trial in which a diet containing 50 p.p.m. dieldrin was fed to female rats. Richardson *et al.* (*13*) determined the concentration of HEOD in the blood of dogs given daily doses of 0.1 mg. dieldrin/kg. for 128 days. These workers reported a highly signficant relationship between the logarithm of the concentration of HEOD in the blood and the logarithm of the concentration of HEOD in the diet (i.e., a power-function relationship). The implications of such a relationship are discussed below, but it was considered desirable to fit the data to an asymptotic relationship of the form fitted to the human data (see below):

$$C=C_\infty (1-e^{-kt})$$

where C is the concentration at time t, C_∞ is the concentration at $t=\infty$, and k is a constant. A comparison of the goodness of fit of the two relationships is given in table 1.

TABLE 1.—*Comparison of the goodness of fit of the concentration—time relationship for HEOD in the blood of dogs*

Dog No.	C_∞	k	Residual variance— about asymptotic relationship	 about power-function
2	429 (±167)	0.0045 (±0.0020)	0.004574	0.004598
4	126 (±13)	0.0171 (±0.0028)	0.004494	0.00568
6	89 (±25)	0.0119 (±0.0047)	0.01664	0.0206

It will be noted that the residual variance about the asymptotic relationship is smaller than that about the power function. For the reasons discussed below it is considered that the asymptotic function is the more appropriate relationship.

Keane and Zavon (*14*) studied the concentration of HEOD in the blood of dogs which had been given 1 mg./HEOD/kg. body weight/day for 5 days, followed by 0.2 mg. HEOD/kg./day for a further 54 days. They concluded that the concentration of dieldrin in the blood of all the animals remained very constant during the last 53 days of the trial, that the ratio of the concentrations of dieldrin in the fat to that in the blood remained relatively constant for each dog during the experiment, and that a storage equilibrium existed during the latter part of the trial. The validity of some of the conclusions of these authors is doubtful,[1] but these doubts do not affect the general conclusion given below.

The experiment in which HEOD was given daily to volunteers for 2 years gave (*9, 10*) results which have been analyzed in detail (*10*) and shown to be consistent with an asymptotic relationship.

The concentration of HEOD in the blood of dogs tends to decline after reaching a maximum (unpublished results, Tunstall Laboratory).

Conclusion: The concentrations of DDT and HEOD in the tissues of rats, dogs, and man do not increase in a rectilinear manner as the time of ingestion increases; the concentrations approach an upper limit, or asymptote, characteristic of the daily dose. If the exposure is continued for a sufficient period of time there are indications that the concentrations in tissues tend to decline.

(iii) Relationships between the concentrations in various tissues—DDT: It appears from figure 10 of Hayes' review (*2*) that the concentrations of DDT in the various tissues are correlated.

Dieldrin: Significant correlations were found between the concentrations of HEOD in the blood and other tissues of rats (*7, 12*) (see fig. 10), dogs (*7*) and man (*9, 10*). Keane and Zavon (*15*) determined the concentrations of dieldrin in the blood and body fat of dogs given daily doses of dieldrin and found a significant correlation between them. The results of Deichmann *et al.* (*12*) also show that the concentrations of dieldrin in body fat and liver are correlated with that in the blood (see fig. 11). These correlations are implicit in the relationships of type I and II discussed above and their explicit demonstration is, in that sense, an example of redundant information. Heuristically, however, it is convenient to discuss these correlations explicitly. The quantitative relationships between the concentrations in the various tissues are also of direct practical interest.

Conclusion: The concentrations of HEOD in the blood are significantly correlated with those in the other tissues and it is probable that corresponding correlations exist in the case of DDT.

(iv) Relationship between concentrations in tissues and time since exposure ceased—DDT: The concentration of DDT in the adipose tissue of monkeys (*3*), and rats (*11*), and cows (*16*) has been found to decline when exposure ceased. The change in concentration is not related to time in either a linear or exponential manner (see figs. 12,

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[1] For example, Keane and Zavon (*14*) state that "the concentration of dieldrin in the blood of all the animals remained very constant from day 7 to day 59. An examination of the results given in table 2 of their paper shows that there was a significant increase in the concentration of dieldrin in the blood during this period:

$$c_{\text{Blood}} = \underset{(\pm 0.016)}{0.078t} + 9.70$$

The slope of the line deviates significantly from zero, and hence the concentration was increasing during the period of the experiment.

13, and 14) and McCully (*16*), for example, suggested that the power function could be fitted to their results. That their results could be fitted to such a relationship is quite probable, but a more plausible relationship is one involving two exponential terms; this type of relationship is shown in figure 14. The decline in the concentration of DDT in the tissues when exposure is terminated implies that DDT is being lost from the body, either as unchanged DDT or as metabolites. Studies of the concentration of DDT or its metabolites in excreta are therefore relevant to this topic.

From the results obtained by Durham *et al.* (*3*) in their study of DDT storage in Rhesus monkeys it may be shown (see fig. 15) that the concentration of DDT and DDA in the excreta are related to the intake of DDT. As the concentration of DDT in the body fat is also related to the daily dose (see sec. I(i) above) the concentrations of DDT and DDA in excreta are also related to the concentration of DDT in the fat. The results of Hayes *et al.* (*4*) and Durham *et al.* (*17*) indicate that the concentration of DDA in the urine of man is also a function of exposure (see figs. 16 and 17).

Dieldrin: The concentrations of HEOD in the tissues of rats decline during the postexposure period (*18*). There are differences in the rates of change of the concentrations in the various tissues (see figs. 18 and 19), and the following empirical relationships, for example, have been derived from the experimental observations:

$$C_{\text{Fat}} = 13.5 \exp(-0.067\,t)$$
$$C_{\text{Liver}} = 0.71 \exp(-0.54\,t) + 0.233 \exp(-0.068\,t)$$

The concentration of HEOD in the blood of workmen has been found to decline when their industrial exposure to aldrin and dieldrin was terminated (*19*) (see fig. 20), and the mean concentration of HEOD in the blood of volunteers who had been given known daily doses of HEOD was also found to have decreased significantly during the 8-month postexposure period (*10*).

The results obtained by Korte and his coworkers in studies of the metabolic fate of ^{14}C-labeled cyclodiene insecticides (*20*) showed that metabolites of these compounds occurred in the excreta of rats and rabbits. In one experiment rats were given ^{14}C-aldrin daily for 12 weeks, and it was found that after about 8 weeks the total amount of ^{14}C-activity in the excreta was equal to the daily administered dose (*21*). It was also found that the majority of the activity was in the form of hydrophilic metabolites. These results, in which the activity eliminated per day eventually balanced the amount administered per day, are the obverse of the approach of the concentrations of

HEOD in the tissues to upper limits, and are analogous to the results obtained in relation to the excretion of DDA by subjects treated with DDT.

Conclusion: The concentrations of DDT and HEOD in body tissues decline when exposure ceases; the relationship between the concentrations in tissues and time during the postexposure period depends upon the compound and the tissue: the declines in the residues of DDT in the body fat of rats, monkeys, and steers appear to deviate significantly from a simple exponential decline, whereas HEOD residues in the body fat of rats do so decline. The residues in HEOD in the liver and blood of rats can be fitted to a relationship involving two exponential terms.

(v) Dynamics of other organochlorine insecticides or related compounds: In surveys of the occurrence of organochlorine compounds in human adipose tissue it has been found that, apart from pp′-DDT and HEOD, residues of pp′-DDD, pp′-DDE, isomers of benzene hexachloride (mainly β and γ), and heptachlor epoxide are also present.

The dynamics of the behavior of these compounds in man are therefore of interest. Unfortunately, the evidence available is slight, but such studies as have been made indicate that their dynamics are of a similar form to those of DDT and HEOD. The most important of these other compounds (as regards the dimension of the residues found) are pp′-DDE and β-BHC, and further information on the kinetics of these two compounds in experimental animals and man is desirable.

(vi) Excretion of organochlorine insecticides in milk and eggs: A number of experimental studies, particularly of DDT and HEOD, have been made of concentrations of organochlorine insecticides in cows milk, and of the eggs of chickens and other birds, in relation to the intensity and duration of exposure. Most of the studies have been concerned with determining empirical relationships and the analysis of the results in relation to the dynamics of these compounds has not usually been carried out. However, the results appear to be generally consistent with the four conclusions drawn above from studies of the concentrations in the body tissues of rats, dogs, steers, and man.

II. *Inferences from the empirical relationships.*—In part I of this review particular stress has been placed upon the relationships between the different variables. A number of inferences may be drawn from the relationships between these variables. In addition to these inferences it is desirable to establish, if possible, a theory or model which will provide a rational basis for the relationships and the deductions based on them. Such a model is required if the results of animal trials are to be used in the interpretation of the epidemiological significance of residues in the general population.

(i) Inferences drawn from the empirical relationships between variables: In the case of HEOD it has been shown that the concentrations of this compound in various body tissues are related to the concentration in the blood in both the exposure and postexposure periods, i.e., there is a concerted change of the concentration of HEOD in the blood and other tissues. Such an inter-relationship may be explained in several ways but the most plausible is that there is a reversible interchange of HEOD between the circulating blood and the other body tissues. Such a reversible process is not unexpected in view of the physico-chemical properties of HEOD, and is analogous to the partitioning of HEOD between different liquid phases. The other organochlorine insecticides will also partition themselves between different phases, differences between them being reflected in the partition coefficients characteristic of the different compounds.

A second inference may be drawn from the observed decline in the concentrations of organochlorine insectides in the body tissues when exposure is terminated. Some of this decline may arise from elimination of the compounds from the body without chemical change (e.g. small amounts of pp′-DDT and HEOD are found in the faeces). However metabolities of DDT and HEOD, for example, have also been found in excreta. This indicates that they undergo biochemical conversion in the body. The rate of conversion at a site(s) of reaction may be expressed in general terms:

Where n is the order of the reaction with regard to the substrate molecule. The concentration of the substrates, in relation to whole tissue, are very low (about $10^{-9}M$ or lower), it is probable that the concentrations at the site(s) of action are also very low. In these circumstances a zero-order reaction (i.e., $n=o$, in which case the rate of metabolism is independent of the concentration) is unlikely. The simplest rate equation is that corresponding to a first order reaction ($n=1$), and the rate of reaction in this case is given by:

$$\frac{dc}{dt}=-kc$$

It is easily shown that this corresponds to an exponential relationship between the concentration *at the site(s)* of reaction and the time since entry of HEOD to the site of reaction was terminated:

$$C'=C_0\exp(-kt)$$

where C' is the concentration at time t and C_0 is the initial concentration at the site(s) of reaction.

The concentration of substrate at the site of reaction when there is a continuing steady rate (α) of injection of the substrate may be derived by integration of the relationship:

$$\frac{dc}{dt}+kc=\alpha$$

$$\text{whence: } C=\frac{\alpha}{k}\,(1-e^{-kt})$$

(it has been assumed, for simplicity, that the concentration of the substrate at the site of reaction was zero before the ingestion of the substrate at a known rate).

With continuing injection of substrate, i.e. as $t\rightarrow\infty$, the concentration at the site(s) of reaction approaches a finite upper limit of asymptotic $(=\frac{\alpha}{k})$. This treatment may be generalised to the case:

$$\frac{dc}{dt}+kc^{n}=\alpha$$

and in all cases for $n>0$, it can be shown that the concentration at the site(s) of reaction approaches a finite upper limit $\sqrt[n]{\frac{\alpha}{k}}$ as time increases.

Unfortunately we are unable to measure the concentrations of substrates at the site(s) of reaction; only the concentrations in tissues or subcellular fractions can be measured. Consequently these inferences regarding the concentrations at the site(s) of reaction are of academic interest *unless we make some other assumptions.*

(ii) The compartmental model and its application to the pharmacokinetics of organochlorine insecticides: The compartmental model has been used, with great success in the study of the dynamics of drugs, lipids, metallic ions, etc. and there appear to be no *a-priori* reasons for not applying such a model to the organochlorine insecticides (*22, 23, 24*).

The basic concepts of the model are as follows. The animal body is considered to consist of an infinite number of infinitesimal elements of volume. Collections of elements form sets, the defining property of each set being that within a set any change in the chemical potential of a substrate in any element of that set results in an instantaneous and equal change of chemical potential of the substrate in all other elements (note that chemical equipotential does not entail equal concentrations of the substrate in all the elements of a set). Such a set is called a compartment. The volumes of the compartments are assumed to be constant, and the boundary between contiguous sets or compartments is regarded as an interface. Some interfaces allow a reversible movement of substrate molecules between compartments, such movements continuing (and therefore taking a finite time) until the chemical potential in all the compartments has approached the same value. In at least one of the compartments biochemical conversion of the substrate may occur, and the rate of conversion is assumed to be of the first-order. Substrates may be eliminated from the organism via one or more compartments. The simplest model is one in which there is only one compartment, and the equations for the behavior of a substrate in such a system are similar to those given above for the concentration of an insecticide at the site(s) of conversion. With models containing two or more compartments, the compartments may be arranged in series, parallel, or combinations of series/parallel.

The compartmental model has been presented above in general terms, and a more specific model, representative of the behavior of organochlorine insecticides, is as follows.

It was argued above that the experimental results indicate a reversible transfer between the circulating blood and other tissues. This corresponds to the so-called mamillary model consisting of a central compartment with one or more peripheral compartments in parallel with it. Direct transfer of the substrate between peripheral compartments cannot be excluded on *a priori* grounds, but the simplest multicompartmental models are those in which such interchange does not occur. A two-compartmental model has been suggested for HEOD in the rat and the experimental results are in good agreement with the predicitions of this model (*18*). The results of studies of the decline of the concentration of DDT in the body fat of rats, monkeys and steers differ from those obtained with HEOD in that a simple exponential relationship does not appear to represent adequately the results (see Figures 12, 13 and 14). More detailed studiess of this point are required, but if there is a real departure from a simple exponential decline it is tempting to speculate that this may arise from DDT in the fat cell behaving as if the latter contained two compartments. Results of studies of the metabolism of lipids in the fat cell have been interpreted on such a basis (*25*).

The compartmental model, in the form proposed above for organochlorine insecticides, is very flexible, even with the contraints used in that model. One or more of the contraints, e.g. constancy of the volumes of compartments or of the partition ratio between peripheral compartments and the central (blood) compartment, may be unrealistic in some circumstances. Thus, in a rapidly growing animal the compartmental volumes are obviously not constant. The phenomenon of enzyme induction may require a change in the assumption concerning the rate of metabolism of a substrate, etc. The mathematical models become very complex in these cases and there is insufficient quantitative data at present to justify further development of the model.

(iii) Pharmacokinetics of organochlorine insecticides in relation to the results of epidemiological surveys: The results of studies of the dynamics of DDT and HEOD in experimental animals (including man) have certain implications in regard to the occurrence of residues of organochlorine insecticides in the general population:

1. The concentrations of these compounds in blood or adipose tissue may be used as indices of total body burden, and of exposure to these compounds.

2. continuing exposure to these compounds will not result in a continuous arithmetic increase of their concentration in body tissues. The upper limit of storage in each tissue for a particular exposure, and the time required to approach this upper limit, are characteristic of each compound[*],

3. changes in the absolute or relative sizes of the compartments in the human body will result in changes in the concentrations in these compartments,

4. when the level of continuing exposure is reduced the concentrations in the body tissues decline; the rates of decline are again characteristic of each compound.

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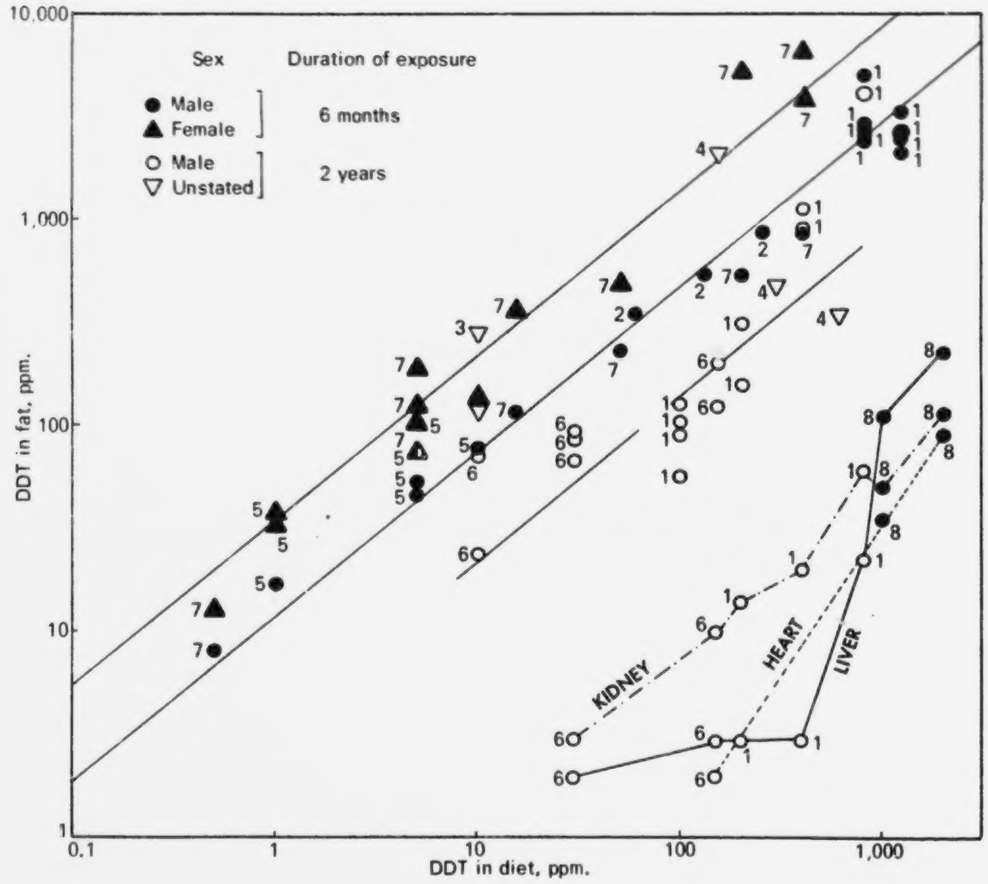

FIGURE 1.—*Storage of DDT in the tissues of rats fed diets containing different concentrations of that compound (based on Figure 10 of Hayes' review*[2]*; the numbers refer to literature citations in the review).*

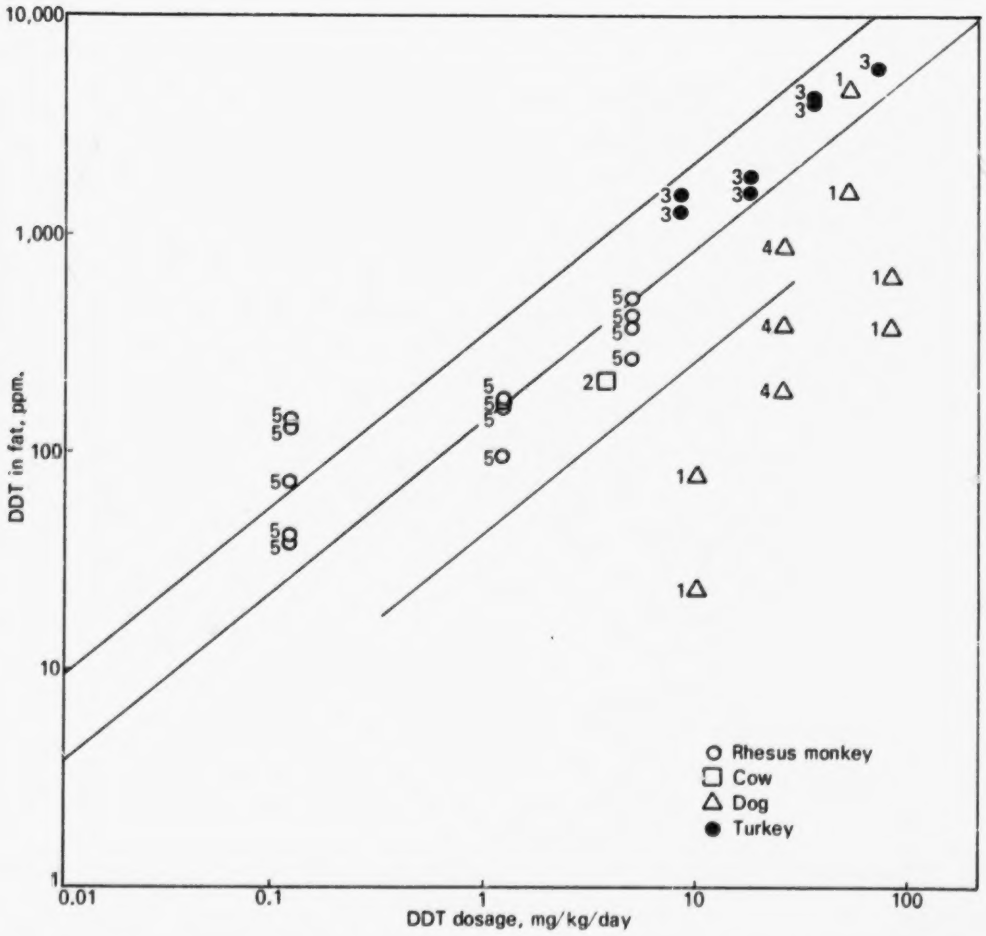

Figure 2.—*Storage of DDT in the adipose tissue of several species of animals given different daily doses of that compound (based on Figure 11 of Hayes' review [2]; the numbers refer to literature citations in the review).*

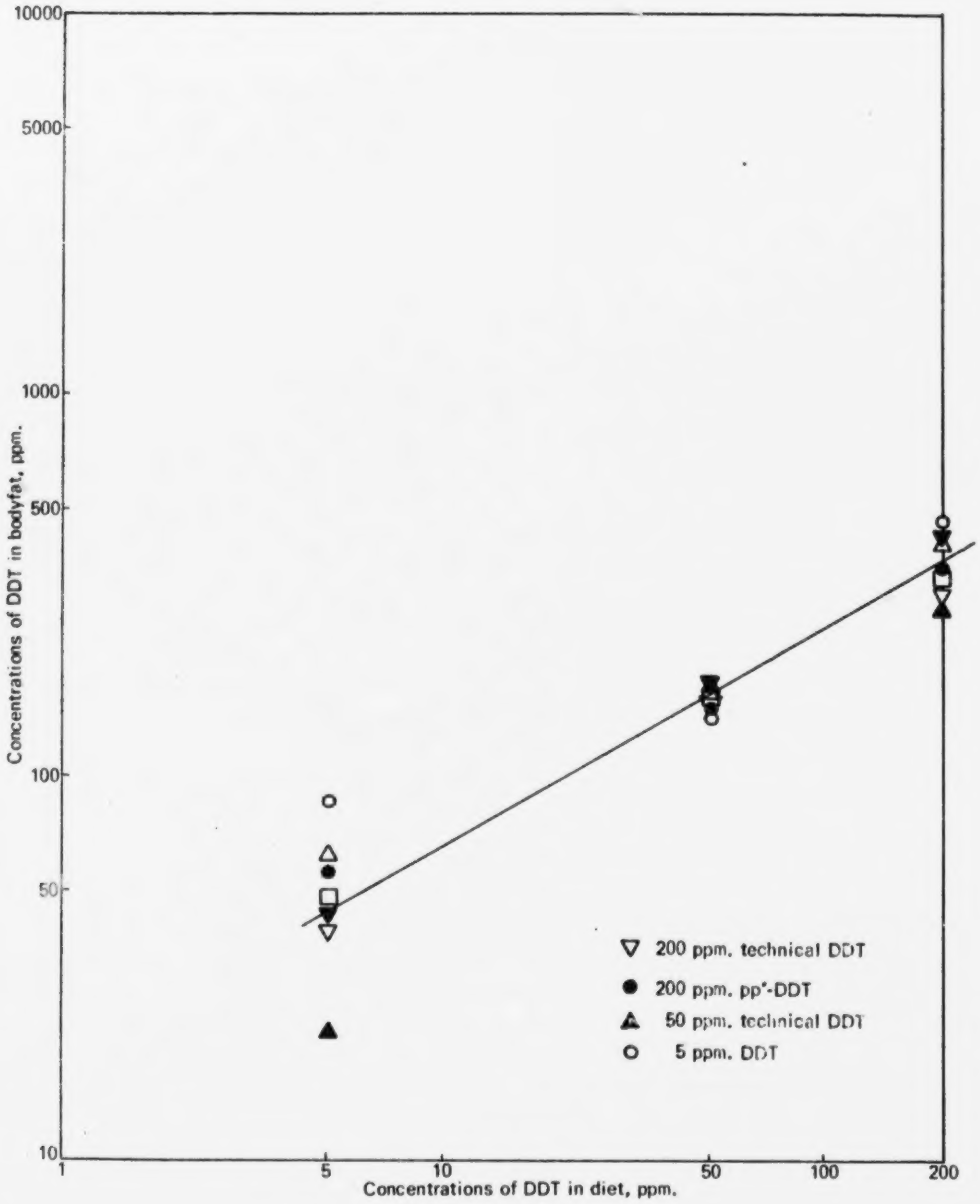

Figure 3.—*Relationship between the concentration of DDT in the bodyfat of rhesus monkeys and the concentration of DDT in the diet (based on Durham et al*[3]*).*

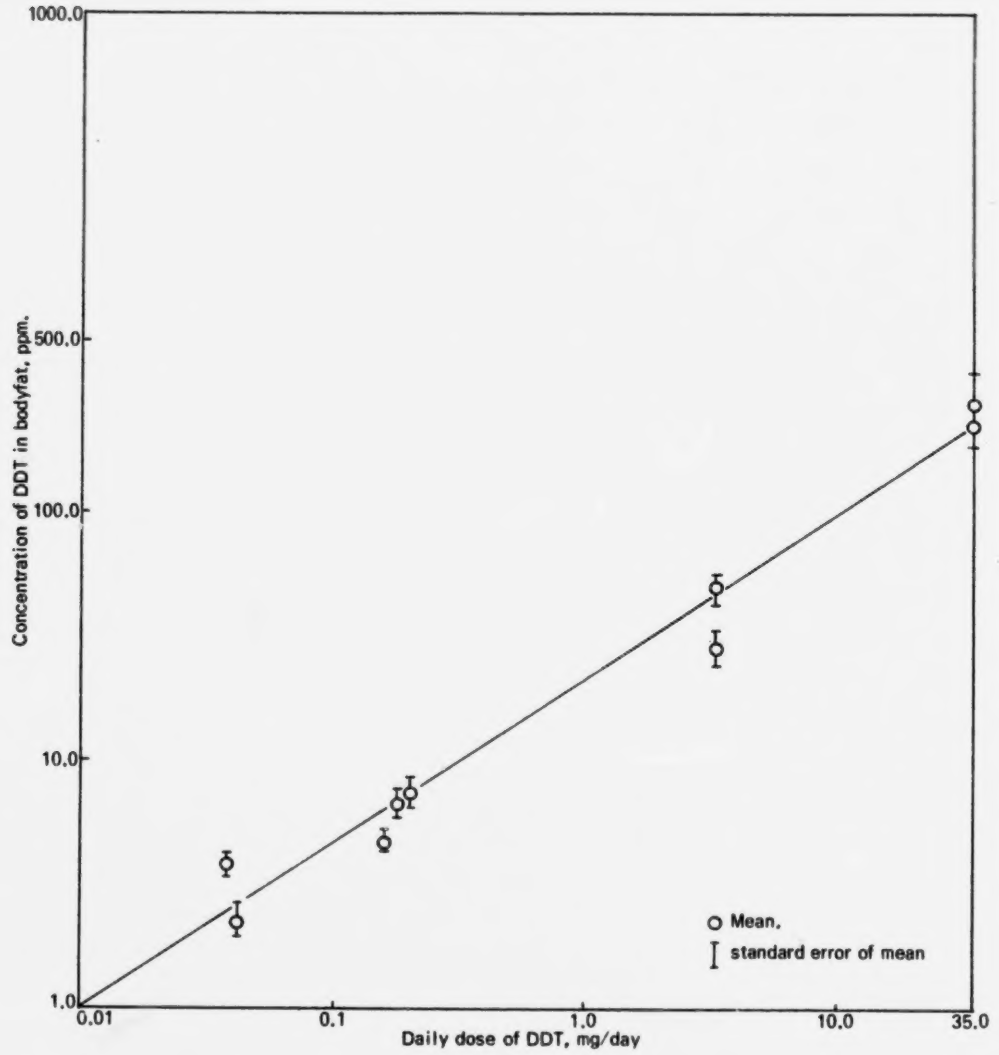

FIGURE 4.—*Relationship between the concentration of DDT in the bodyfat of man and the daily dose of that compound (based on Hayes et al*[4] *and Durham et al*[6]*).*

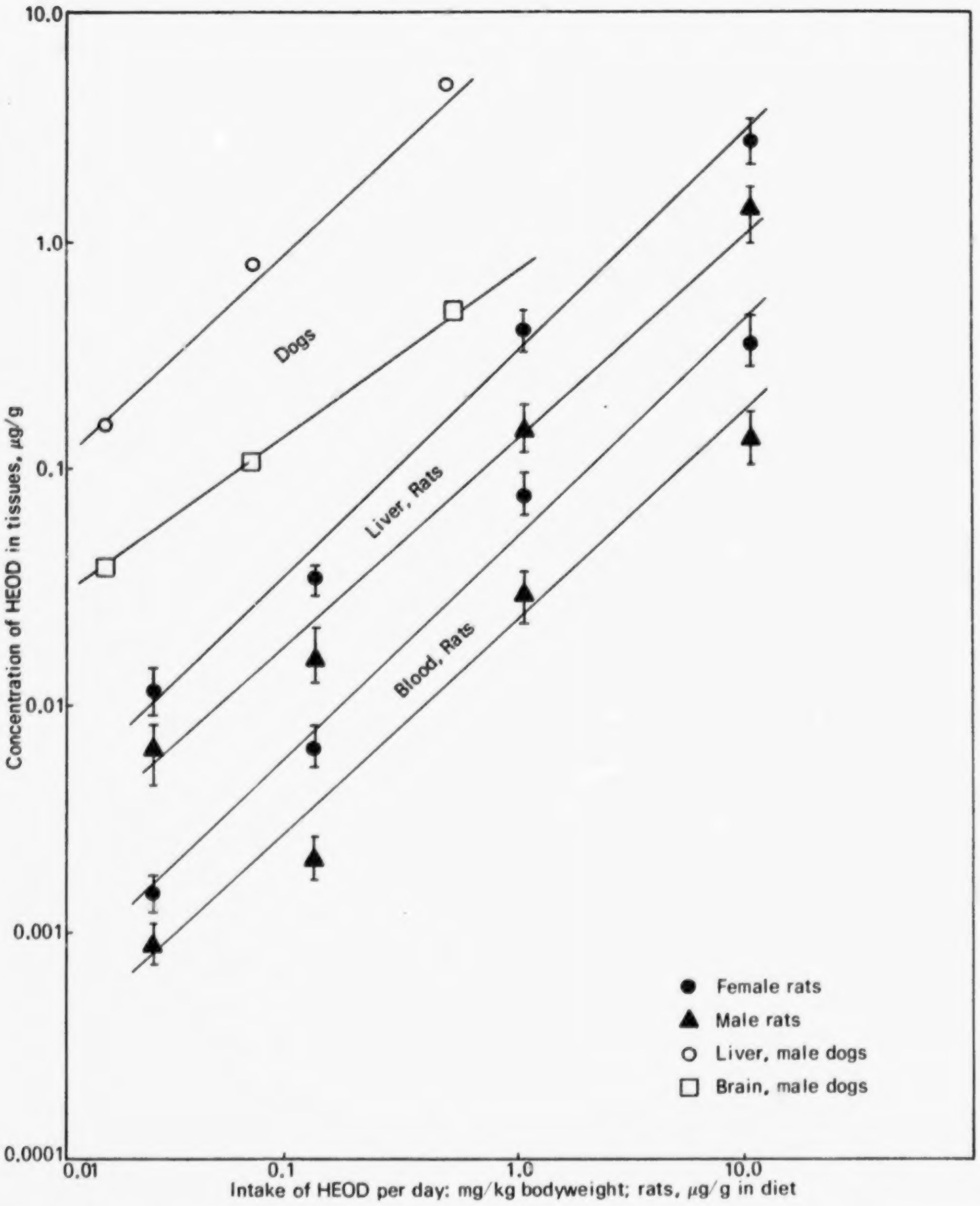

Figure 5.—*Relationship between the concentration of HEOD in the tissues of rats and dogs and the daily intake of that compound (based on Walker et al*[7]*).*

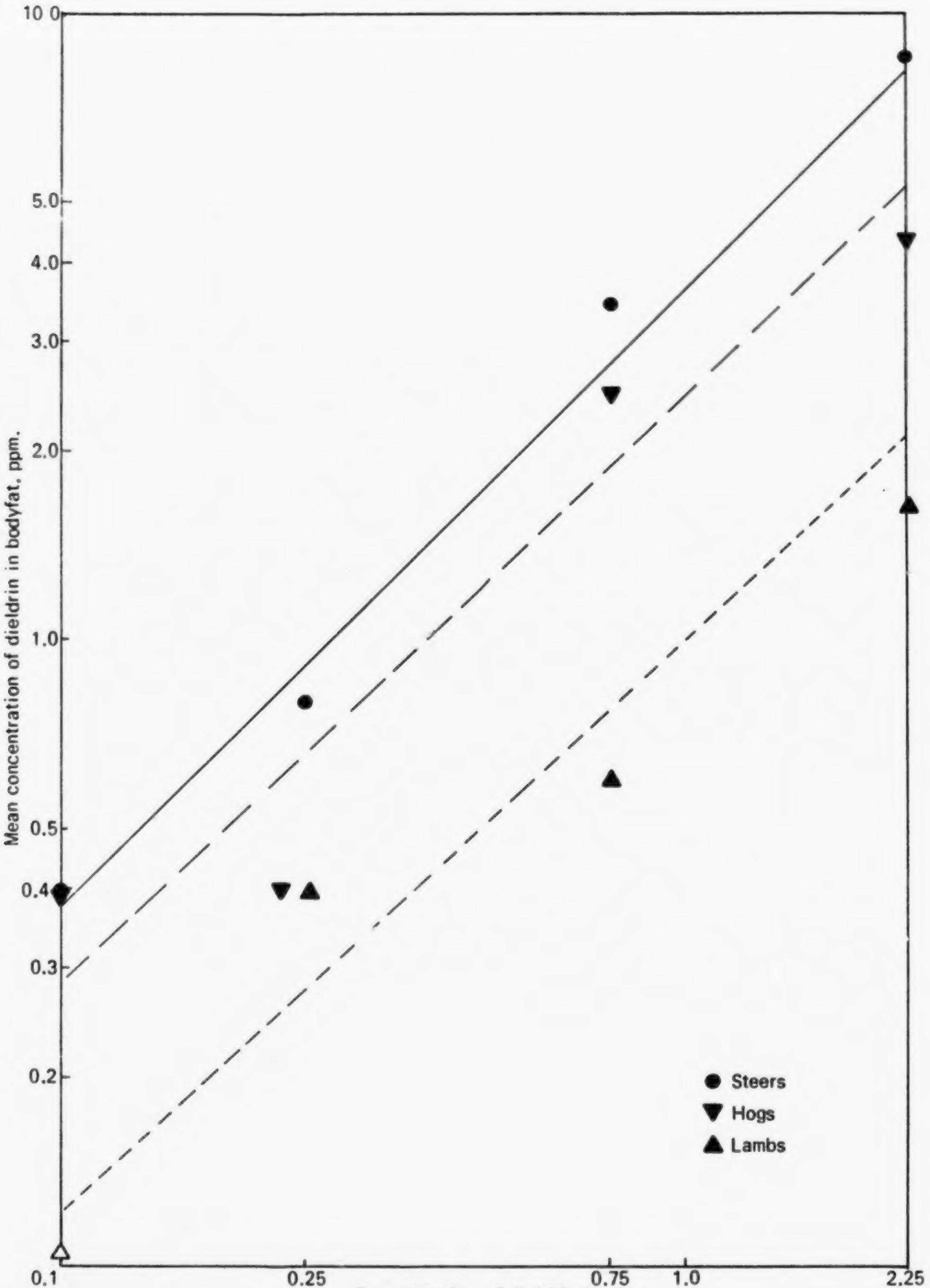

FIGURE 6.—*Relationship between the concentration of dieldrin in the bodyfat of steers, hogs and lambs, and the concentration of dieldrin in the diet (based on Gannon et al*[8]*).*

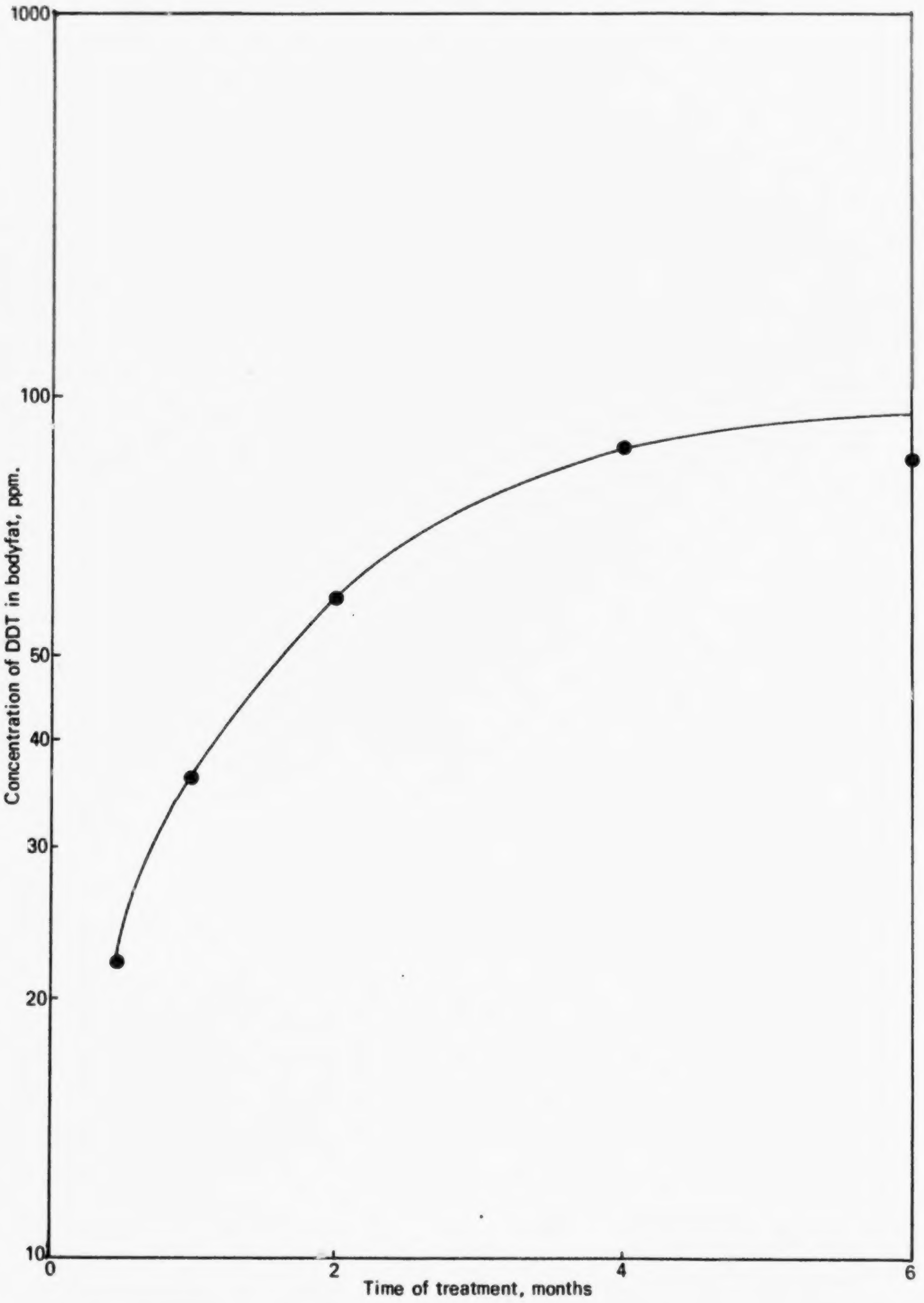

Figure 7.—*Increase of the concentration of DDT in the bodyfat of male rats fed 5 ppm. technical DDT in their diet for 6 months (based on Ortega et al*[11]*).*

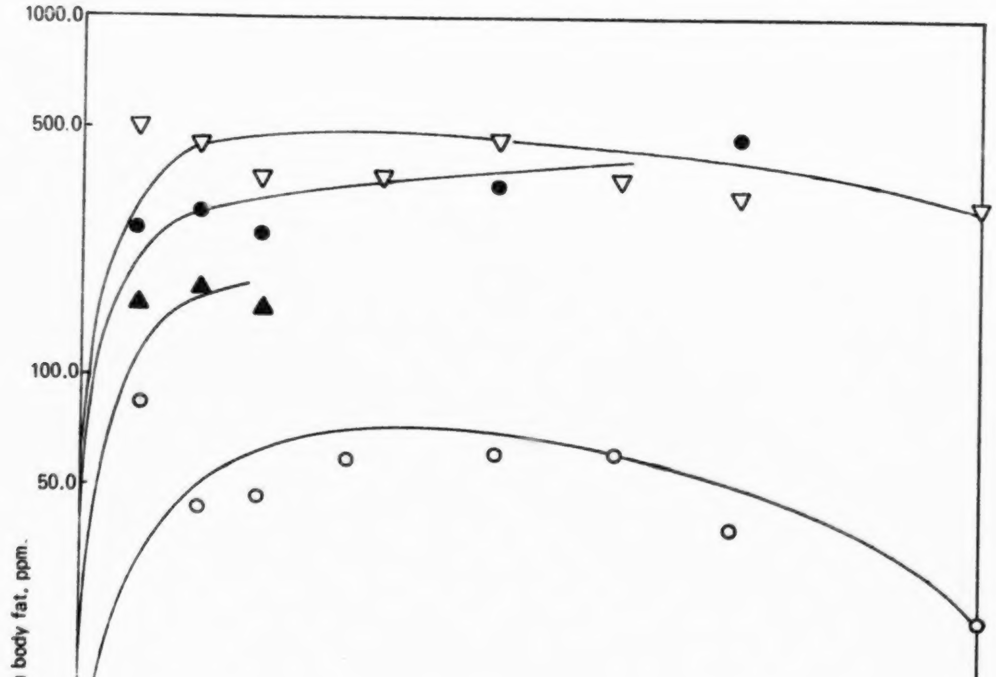

Figure 8.—*Increase of the concentration of DDT in the bodyfat of rhesus monkeys with continuing exposure to DDT (based on Durham et al*[3]*).*

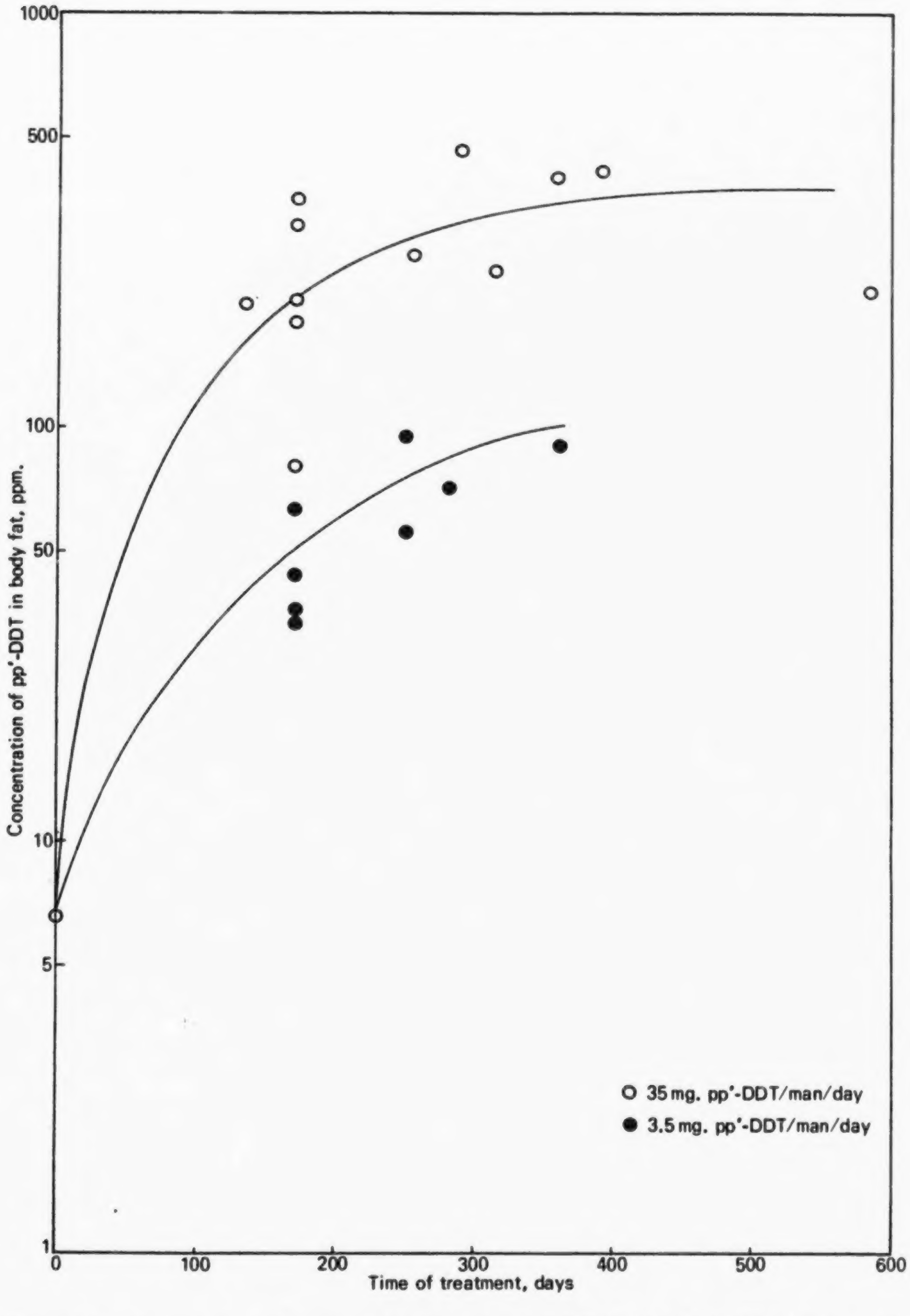

Figure 9.—*Increase of the concentration of pp'-DDT in the bodyfat of men with continuing intake of pp'-DDT (based on Hayes et al*[4]*).*

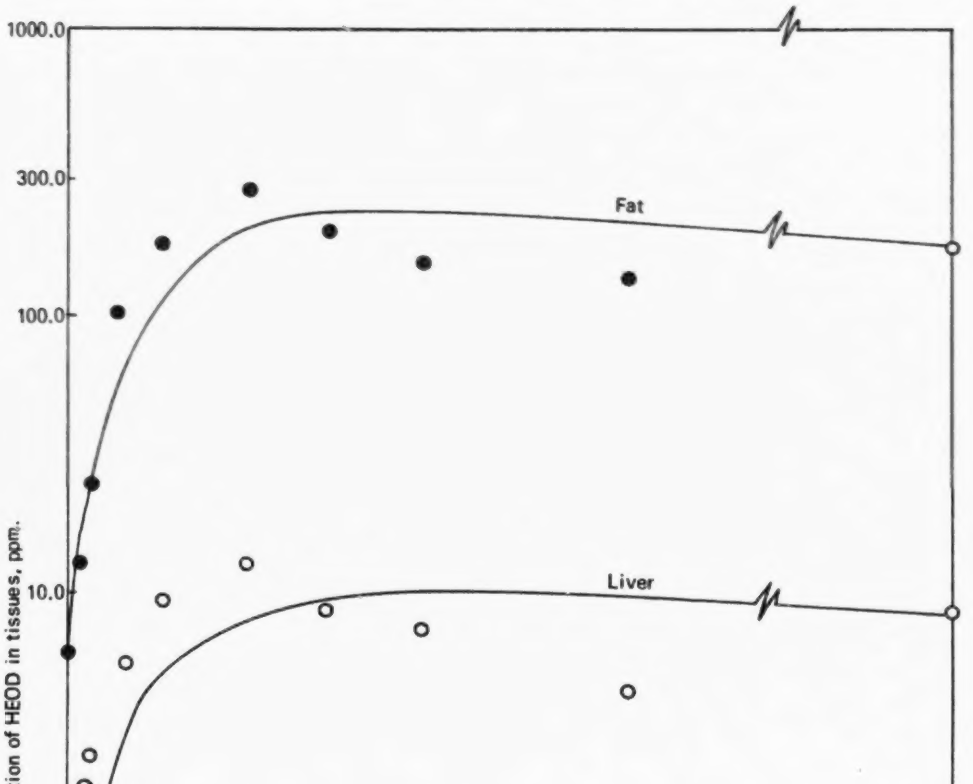

FIGURE 10.—*Relationship between the concentration of HEOD in the tissues of female rats and the time of ingestion of a diet containing 50 ppm. of HEOD (based on Deichmann et al* [12]*).*

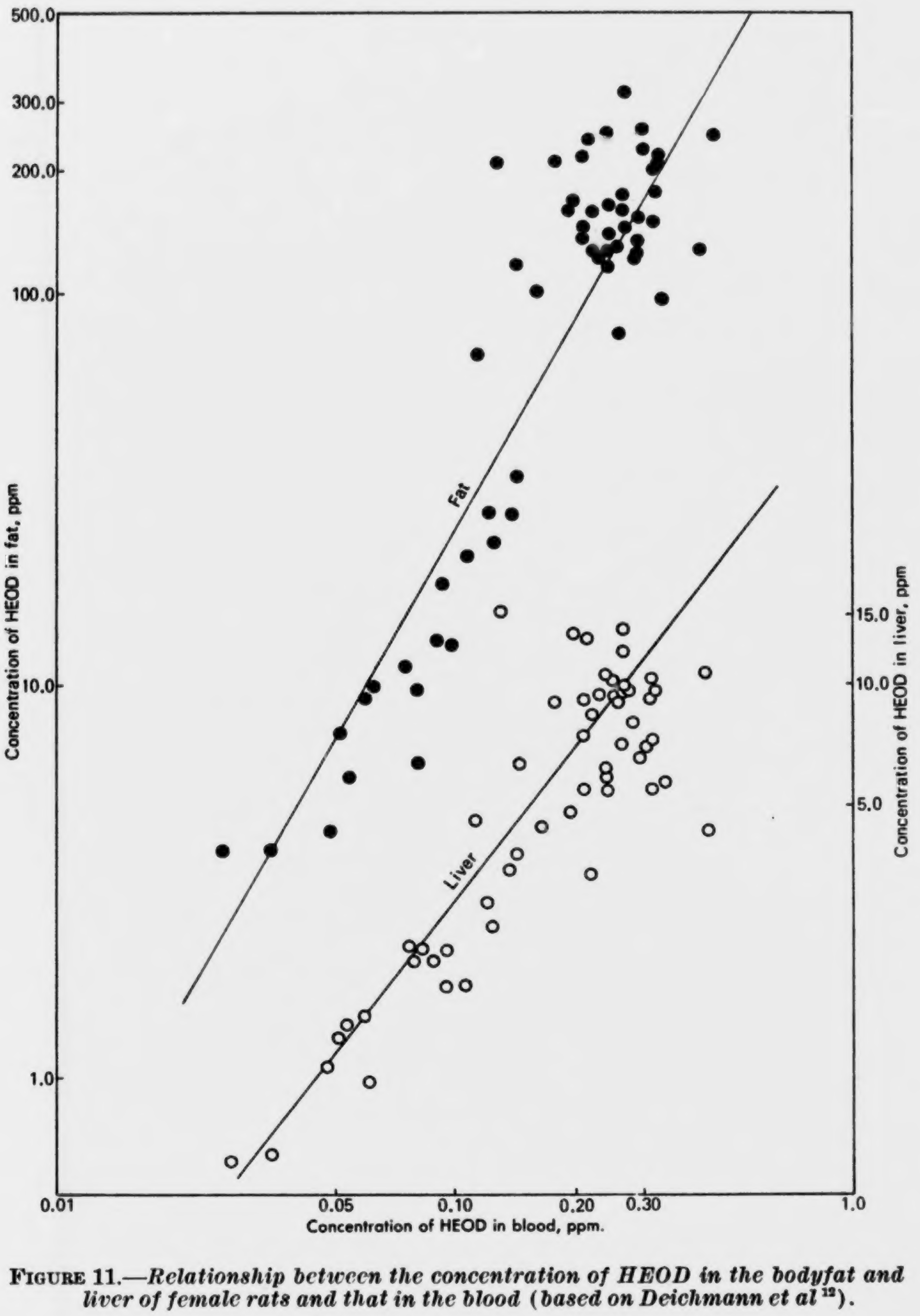

Figure 11.—*Relationship between the concentration of HEOD in the bodyfat and liver of female rats and that in the blood (based on Deichmann et al*[12]*).*

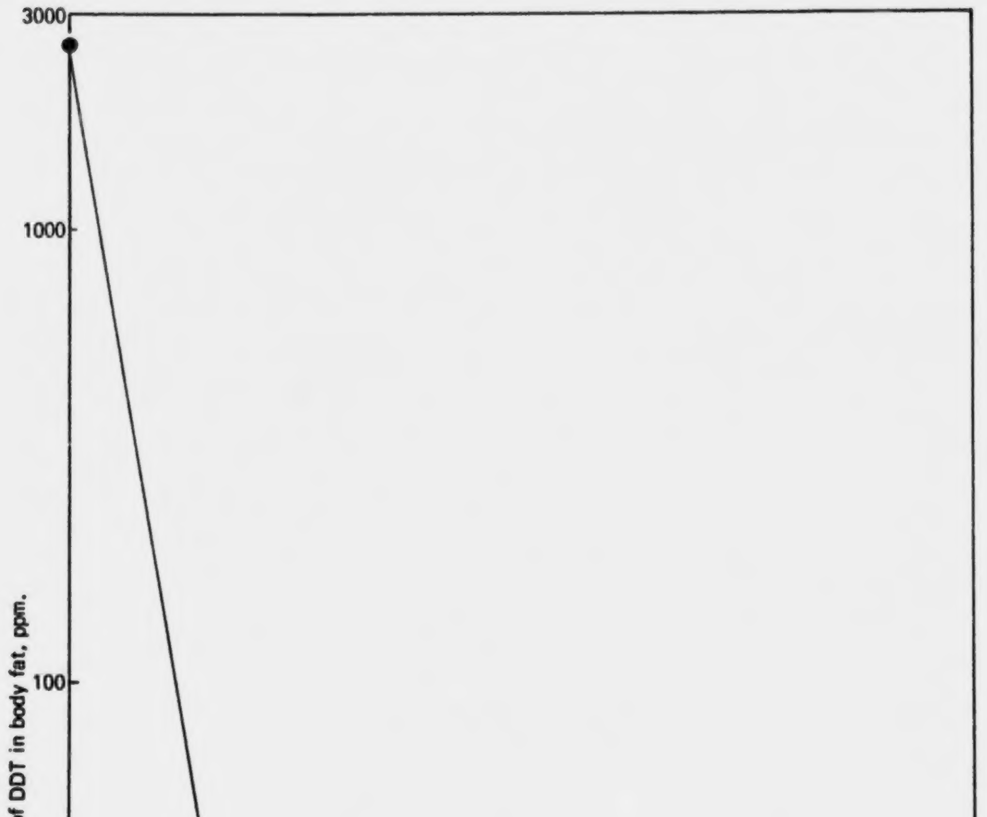

FIGURE 12.—*Decline of the concentration of DDT in the bodyfat of rhesus monkeys after exposure has ceased (based on Durham et al*[3]*).*

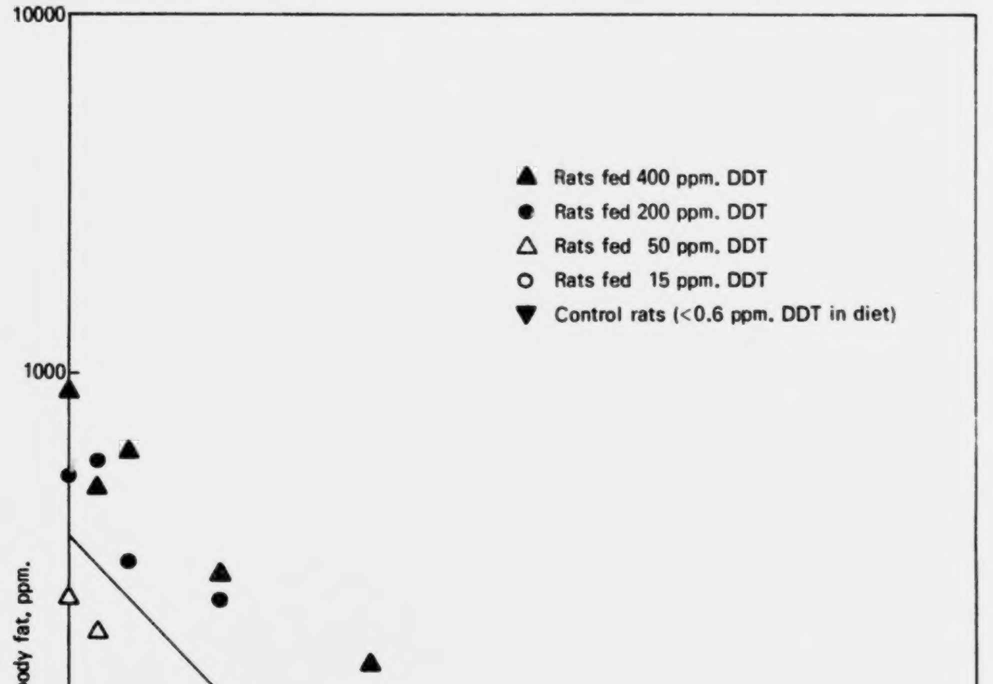

FIGURE 13.—*Decline of the concentration of DDT in the bodyfat of male rats during the post-exposure period (based on Ortega et al[11]).*

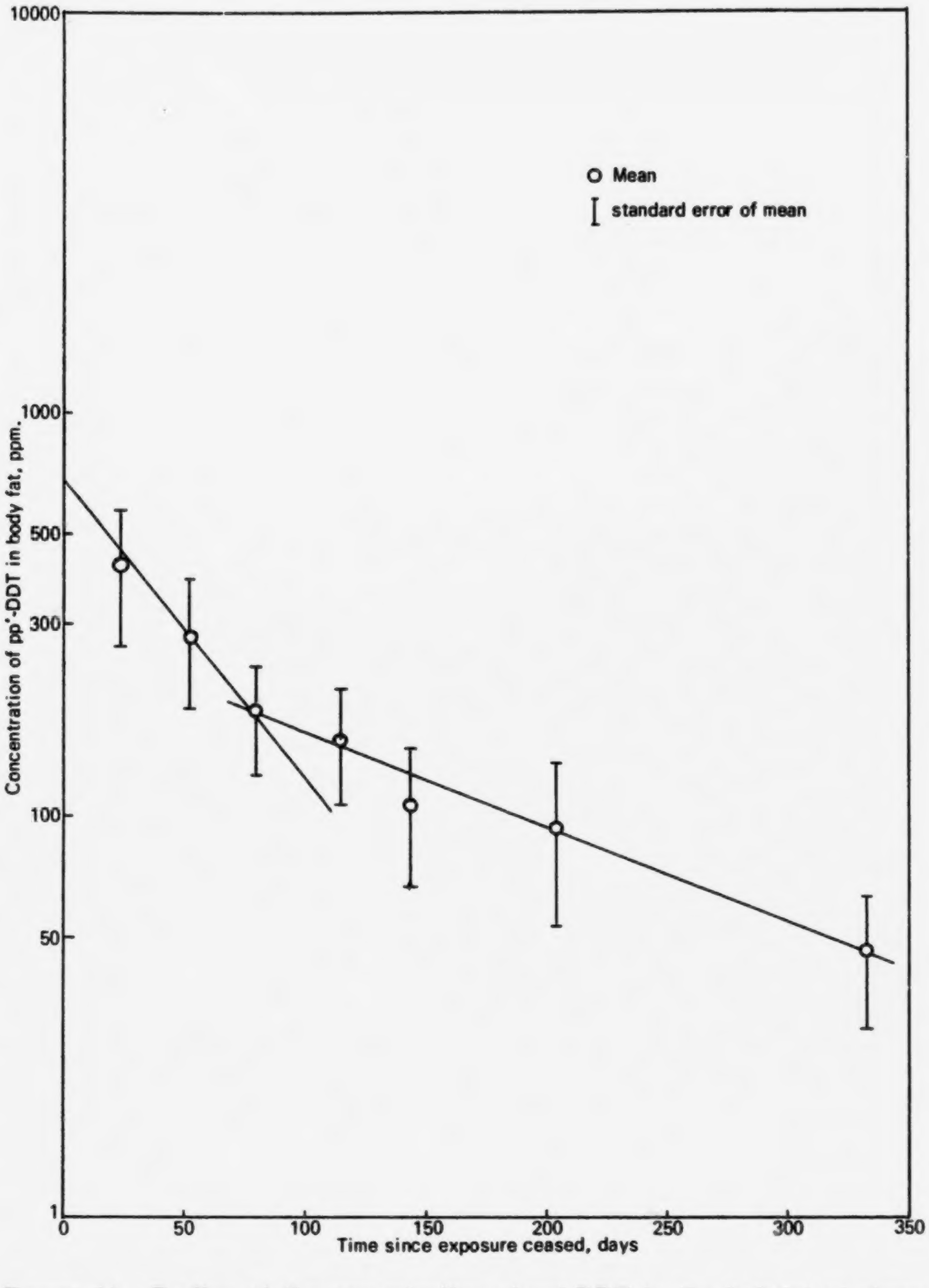

FIGURE 14.—*Decline of the concentration of pp'-DDT in the bodyfat of steers after exposure has ceased (based on McCully et al*[16]*).*

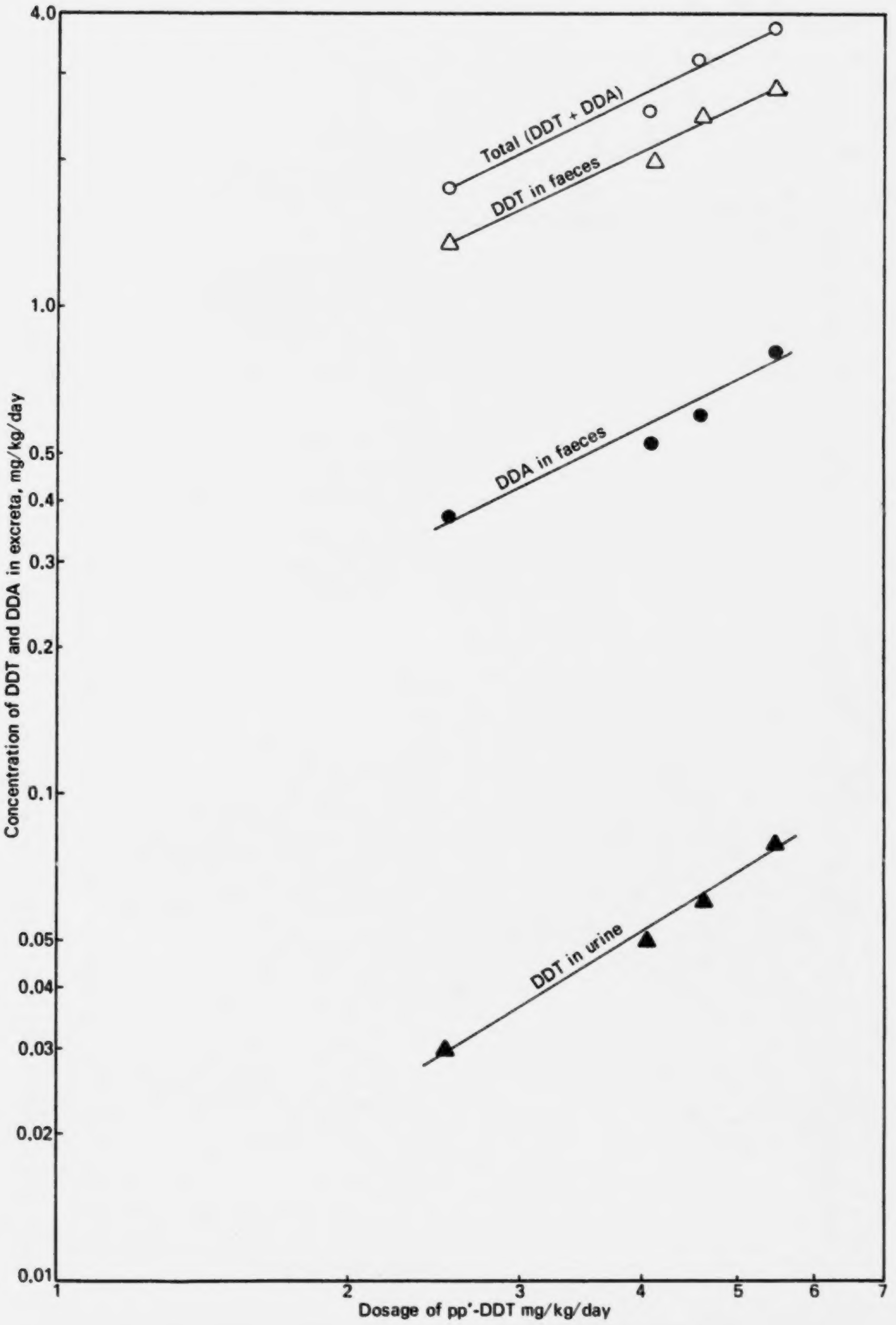

FIGURE 15.—*Relationship between the concentration of DDT and DDA in the excreta of rhesus monkeys and the daily dose of DDT (based on Durham et al*[3]*).*

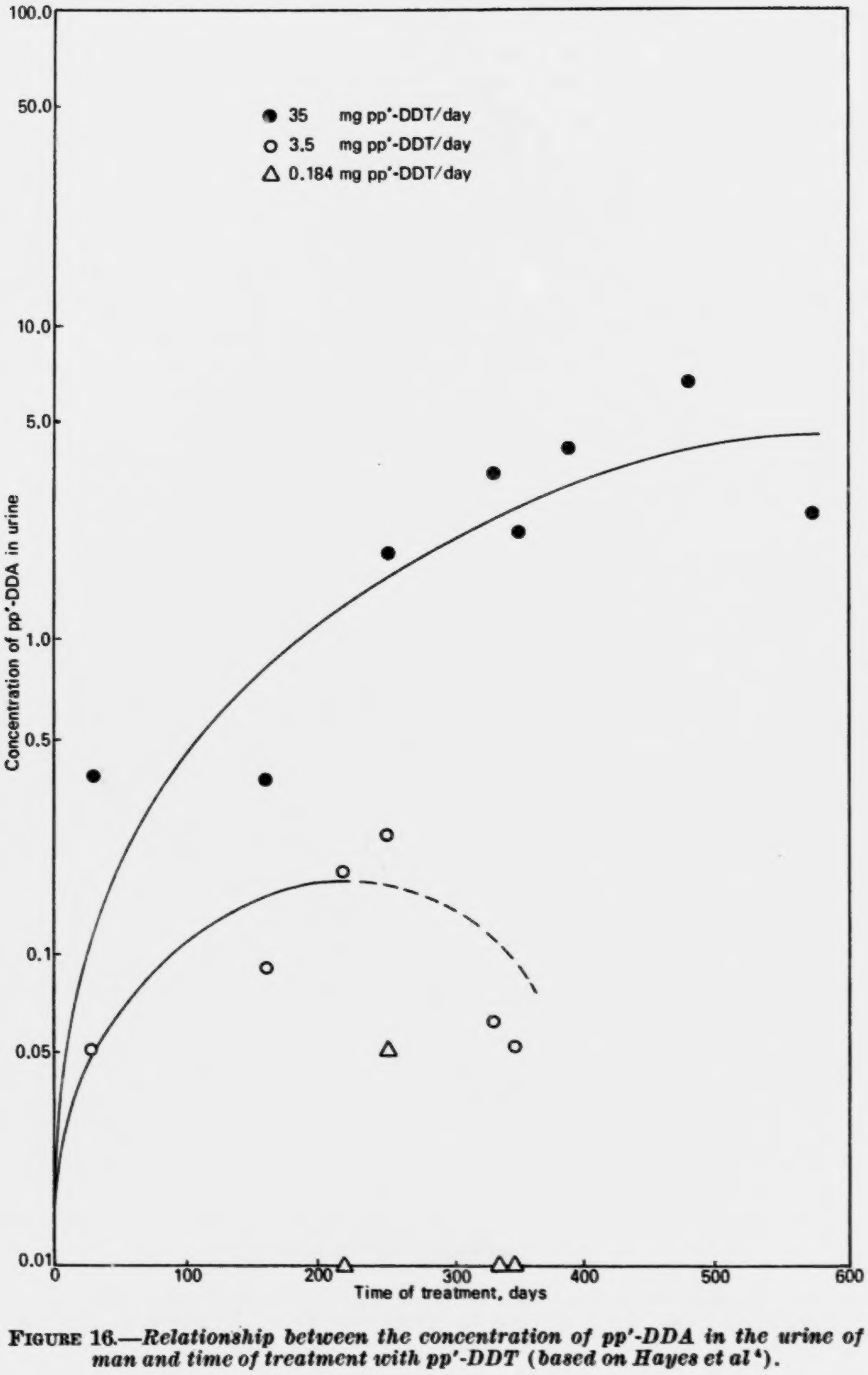

Figure 16.—*Relationship between the concentration of pp'-DDA in the urine of man and time of treatment with pp'-DDT (based on Hayes et al[4]).*

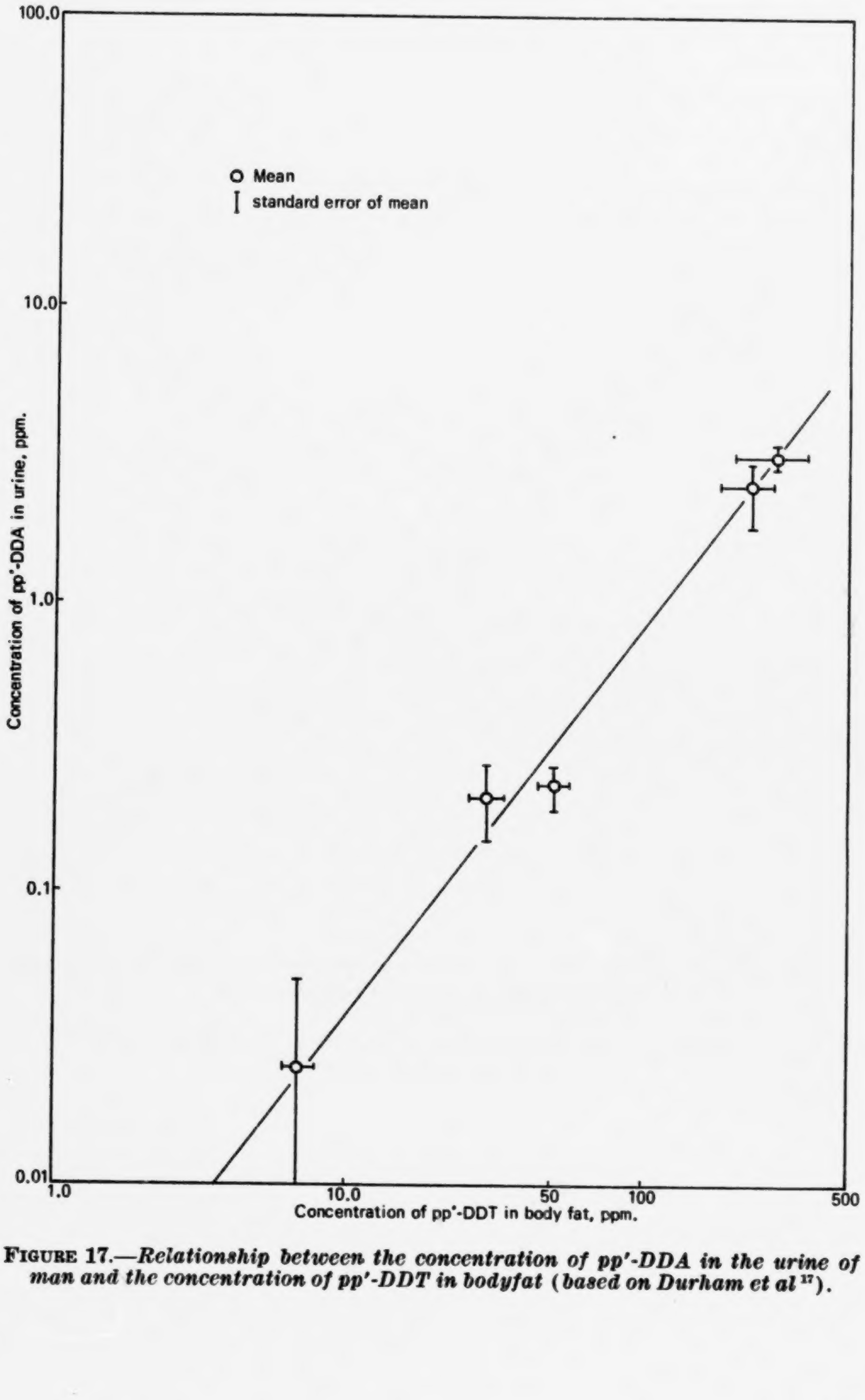

FIGURE 17.—*Relationship between the concentration of pp'-DDA in the urine of man and the concentration of pp'-DDT in bodyfat (based on Durham et al*[17]*).*

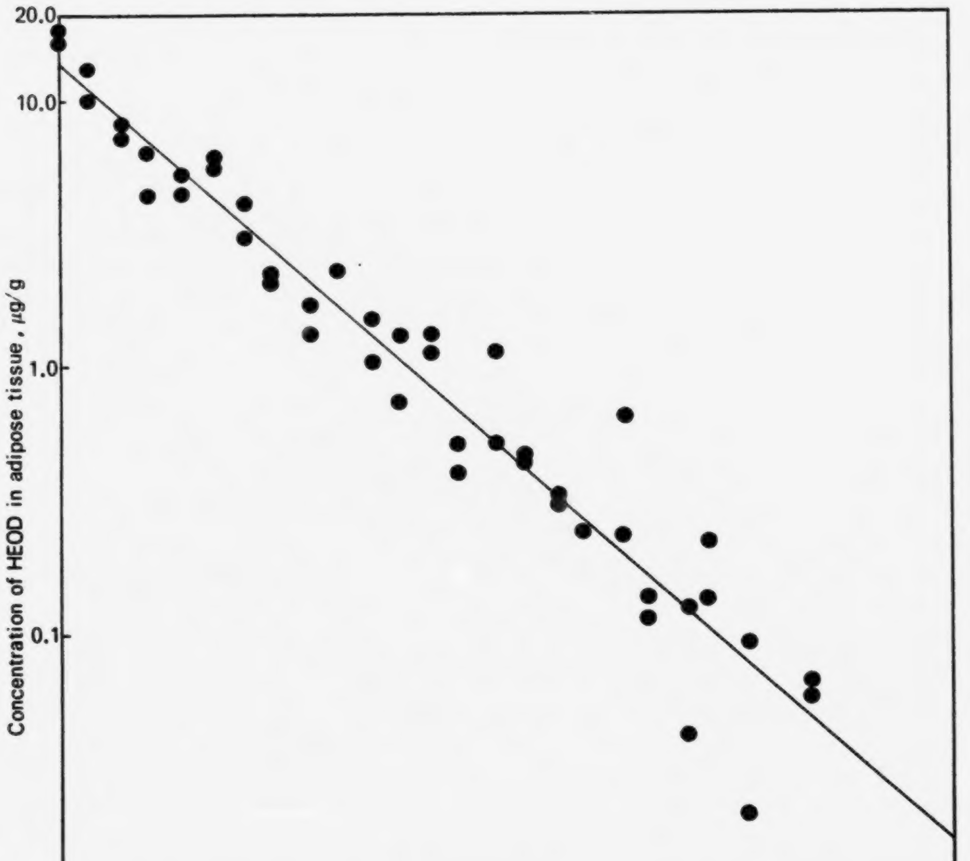

Figure 18.—*Decline of the concentration of HEOD in the adipose tissue of rats during the post exposure period.*[18]

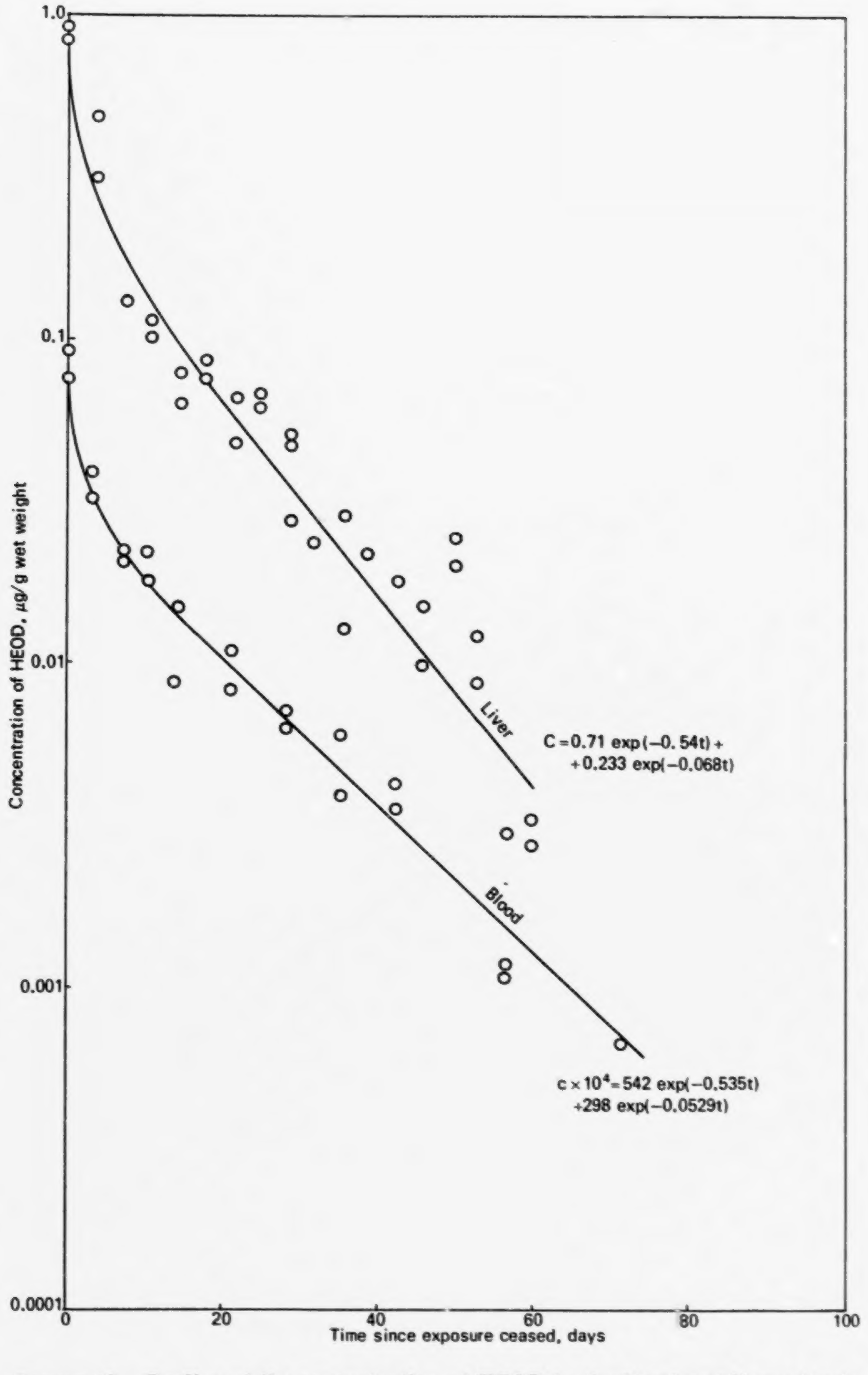

FIGURE 19.—*Decline of the concentration of HEOD in the blood and liver of rats during the post exposure period.*[18]

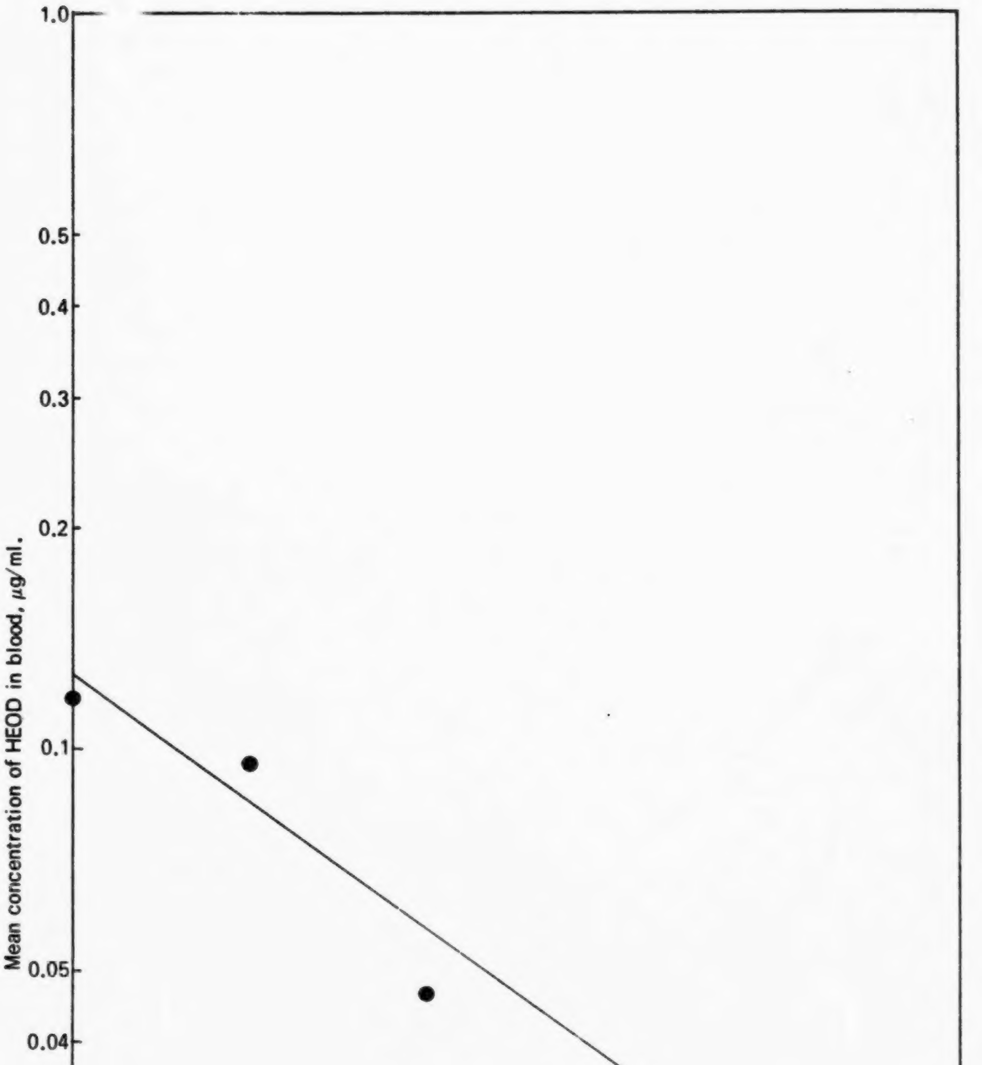

Figure 20.—*Decline of the concentration of HEOD in the blood of workmen after industrial exposure has been terminated (based on Jager[19]).*

In this section, published reports on the relationship between pesticide exposure and human health are grouped according to three major classes of evidence: (1) Controlled human exposures; (2) epidemiological evidence, involving retrospective or planned prospective observations of defined human populations exposed to pesticides, and preferably involving also an unexposed "control" group and (3) clinical case reports, in which one or several human subjects have apparently been made ill as a result of exposure to a single pesticide or a combination of pesticides.

Conclusions reached from these forms of evidence vary in validity and reliability. Evidence based on controlled human exposure is the most reliable of all but obviously relates only to the dosage employed and other conditions of the study. Also, the subjects selected can never adequately represent the wide variety of exposed population groups to which reference was made above.

Clinical evidence is vital to the physician in his efforts to understand the symptomatology of human intoxication, and discover effective courses of therapy. However, this kind of evidence is sometimes the source of unprovable conjectures and allegations about cause and effect relationships.

The epidemiological approach may provide statistically significant evidence of differences in morbidity and mortality patterns between exposed and control groups. This allows investigators to focus upon specific disease entities or syndromes which are particularly prevalent, to identify the "weak link" in the chain of causation, and to plan programs of prevention. Unfortunately, the complexities and cost of study design and execution tend to discourage widespread application of this valuable technique.

Controlled human exposures

Because of the complexities of extrapolation of animal data to humans, the literature cites many examples of human volunteer studies where pesticides have been given or applied to human volunteers. Such persons have received a prescribed dose of pesticide, single or in combination for a prescribed duration of time. The main purpose toxicological doses, or to evaluate the route of absorption or the effect of such variables as protective clothing, temperature, etc. or the characteristics of the pesticide vehicle (dust versus emulsifiable concentrate). In addition, considerable information on the pharmacodynamics of and storage of pesticide has been obtained from several well-conducted studies. Insofar as oral doses of organophosphates are concerned, the works of Rider, J. A. and Moeller, M. C. and their establishment of incipient oral toxicity levels of the more commonly used organophosphates should be especially cited. Utilizing as indicative of minimal toxicity that amount which when ingested over a period of several weeks will produce a symptomless average decrease in cholinesterase activity of 20–25 percent below control values. Doses for parathion, systox, octamethyl pyrophosphoramide (OMPA) and methyl parathion in man were 7.5 mg., 6.75 mg., 1.5 mg., and between 11 and 19 mg.'s per day respectively. (Rider, *et al*, 1967). Hartwell, *et al* (1964) conducted a controlled respiratory exposure study on human volunteers using parathion. Their results indicated that the respiratory route was the more toxic and that ambient temperature was a significant factor in toxicity. Dermal exposures of human volunteers were conducted of Hayes, G. R., *et al*, 1966. Parathion dust and emulsion produced no cholinesterase inhibition when applied to the arms, but when the subjects evolving, excluding the head was exposed a 56 percent decline in plasma values was observed with dust.

Studies in volunteers.—DDT, DDD, methoxychlor, and dieldrin have been studied in volunteers. One of them, DDT, has been tested to learn how much would be dangerous under very adverse conditions of use. All have been explored to learn the safety of repeated doses much larger than those ever contacted by ordinary people and at least somewhat larger than those usually encountered by men who make or mix the compounds.

When it became apparent during World War II that DDT was effective for controlling the vectors of malaria, typhus, plague, and certain other diseases of tremendous military importance, the toxicity of the compound was studied by teams of investigators in several countries. Since the results of animal tests were encouraging, the studies were carried to volunteers to explore the safety of military use and even the effects of accidental ingestion. Following the war, volunteers were studied to learn the effects of daily ingestion of large doses.

Experimental respiratory exposure to DDT.—In order to determine the consequence of frequent and indiscriminate use of DDT, Fennah (1945) inhaled 100 mg./day for a total of 11.5 months. No ill effects were observed. Neal *et al.* (1944) reported almost continuous daily exposure to aerosols sufficient to leave a white deposit of DDT on the nasal vibrissae of the volunteers. This exposure produced moderate irritation of the nose, throat, and eyes. Except for this irritation during exposure, there were no symptoms, and laboratory tests and physical examination, including neurological evaluation, failed to reveal any significant changes.

Experimental dermal exposure to DDT.—In three subjects, experimental dermal exposure to DDT was followed by fatigue, aching of the limbs, anxiety or irritability, and other subjective complaints.

Recovery was delayed a month or more (Wigglesworth, 1945; Case, 1945). In neither instance was there an independent control. Although the dosage was unmeasured, the amounts of DDT absorbed must have been much smaller than those involved in subacute oral tests and very much smaller than minimal single oral doses (6 to 10 mg./kg.) that have led to mild illness. One of the studies involved self-experimentation by one man. A similar but somewhat more severe test on six volunteers produced no toxic or irritant effect at all (Dangerfield, 1946). In view of all other experiments and extensive practical experience, it must be concluded that the illnesses reported by Wigglesworth and by Case were unrelated to DDT.

With the exceptions just mentioned, dermal exposure to DDT has been associated with no illness and usually no irritation (Domenjoz, 1944; Cameron and Burgess, 1945; Dangerfield, 1946; Chin and T'Ant, 1946; Wasicky and Unti, 1944; Draize *et al.*, 1944; Haag *et al.*, 1948; Fennah, 1945). In fact, Hoffman and Lendle (1948) reported that even subcutaneous injection of colloidal suspensions of DDT in saline in concentrations up to 30 p.p.m. caused no irritation. Zein-el-Dine (1946) reported that DDT-impregnated clothing caused a slight, transient dermatitis, but the method of impregnation was not stated and the absence of solvent was not guaranteed. Other more thorough studies of DDT-imgregnated clothing have found it not irritating (Domenjoz, 1944; Cameron and Burgess, 1945).

Chin and T'Ant (1946) applied small pads impregnated with different formulations of DDT to the inner surface of the forearm of 32 volunteers whose cutaneous sensation had previously been measured for a period of 4 weeks. Pads impregnated with all the elements of the formulation except DDT were applied to the corresponding position of the other arm as a control. Powdered DDT and 5 percent solutions of DDT showed little effect. Ten percent and 20 percent solutions in olive oil and petrolatum showed no remarkable effect on sensation of pain, cold or heat, but reduced tactile sensation in most cases so that the minimal pressure which could arouse the tactile sensation was 1 to 2.5 g./cm.^{2} higher than in the control.

Experimental oral exposure to DDT.—Careful study of volunteers who ingested one or a few large doses of DDT have established that 10 mg./kg. is the threshold dosage that leads to significant discomfort in some people but no detectable effect in others. That the observed difference is due to individual variation of the subjects is indicated by the results of accidents in which dosage of 10 mg./kg. (or in a single instance, 6 mg./kg.) led to mild poisoning in some persons but no effect in others (Hsieh, 1954). However, it is also possible that investigators have differed in their evaluation of completely subjective changes attributed by some to dosages of about 3.5 mg./kg.

Velbinger (1947a, 1947b) reported that doses of 250 or 500 mg. of DDT in the form of a suspension or a solution in oil produced no effect except a variable, slight disturbance of the sensitivity of the mouth. Doses of 750 or 1000 mg. in oil solution led to disturbance of the sensitivity of the lower part of the face, uncertainty of gait, malaise, hypersensitivity to contact, cool moist skin, but no change in reflexes. Discomfort reached a peak about 6 hours after ingestion. A dose of 1500 mg. in oil solution produced prickling of the tongue and around the mouth and nose beginning about 2.5 hours after the dose. Disturbance of equilibrium, dizziness, confusion, and tremor of the extremities gradually increased. A peak reaction characterized by malaise, headache, fatigue and delayed vomiting was reached about 10 hours after ingestion. Recovery was almost complete in 24 hours.

Other investigators (Domenjoz, 1944; MacCormack, 1945; Neal *et al.*, 1946) found this same range of doses (250 to 1500 mg.) were without clinical effect. The difference was not associated with failure of absorption, for excretion of the metabolite DDA was measured in connection with one study (Neal *et al.*, 1946) and it was noted that lice were killed when fed on a man 6 and 12 hours after he had ingested 1500 mg. of DDT dissolved in butter (MacCormack, 1945).

It has been noted in the course of tests with volunteers that dilute colloidal aqueous suspensions of DDT are odorless and tasteless (Domenjoz, 1944; Hoffman and Lendle, 1948). Saturated alcoholic solutions of DDT have a weak aromatic taste or rather odor. Some people find these solutions slightly anaesthetic to the tongue (Hoffman and Lendle, 1948). The taste of DDT in vegetable oil is so slight that many persons can not identify capsules containing 0, 3.5 and 35 mg. of DDT when they are presented separately but can arrange them in proper order when one of each is available for comparison.

The possible clinical effects of many repeated doses of DDT were first explored by Fennah (1945). Because of his interest in predicting the results of indiscriminate use, he expressed the exposures in terms of environmental levels rather than as dosage units. The exposures were clearly higher than those ordinarily encountered. In one test, lasting a total of 11.5 months, Fennah daily inhaled 100 mg. of pure DDT and drank water dusted at the rate of 3,240 mg./m.^{2}. Much of the inhaled dust must have been deposited in the upper respiratory tract and swallowed. Later, for 1 month, Fennah ate food all of which had been sprayed at the rate of 2,160 mg./m.^{2} after it had been served. No ill effect of any kind was observed.

Later, studies of DDT in volunteers were designed to explore the details of storage and excretion of the compounds in man and to search for possible effects of doses considered to be safe. In the first of these studies, men were given 0, 3.5 and 35 mm./man/day. These doses, plus DDT measured in the men's food, resulted in dosage levels of 0.0021–0.0034, 0.038–0.063, and 0.36–0.61 mg./kg./day, respectively, the exact value depending on the weight of each individual. Six volunteers received the highest dose of technical DDT for 12 months, and three received it for 18 months. A smaller number of men ingested the lower dose of technical DDT or one of the doses of recrystallized DDT for 12 or 18 months. No volunteer complained of any symptom or showed by the tests used any sign of illness that did not have an easily recognizable cause clearly unrelated to the exposure to DDT. At intervals, the men were given a systems review, physical examination, and a variety of laboratory tests. Particular attention was given to the neurological examination and liver function tests, because the major effects of DDT in animals involve the nervous system and the liver (Hayes *et al.*, 1956). The same result was obtained in a second study in which the same doses were given for 21.5 months and the volunteers were observed for a minimum of 25.5 additional months (Hayes *et al.*, 1961).

In both studies, storage of DDT was proportional to dosage. Men who received p,p′–DDT at the average rate of 0.5 mg./kg./day stored concentrations ranging from 129 to 659 p.p.m. in their fat, with an average of 325±62.2 p.p.m. Those receiving technical DDT at the same rate stored 105 to 619 p.p.m. with an average of 281±79.5 p.p.m. The results were statistically indistinguishable in the two studies. When dosing was started, the urinary excretion of DDA increased rapidly at first and then more gradually until a steady state was reached in 6 to 8 months in different groups of men. A longer period (18.8 to 21.5 months) was required to approach a steady state of storage in fat. The greater storage of p,p′–DDT was matched by lesser excretion; during the steady state the average urinary concentration of DDA derived from p,p′–DDT was 1.88 p.p.m., while that from technical DDT was 2.98 p.p.m. During the latter part of the dosing period, it was possible in the two groups receiving recrystallized and technical DDT at the rate of 35 mg./man/day to account for an average of 13 percent and 16 percent, respectively, of the daily dose in terms of urinary DDA. The excretion of DDA was relatively constant in each individual, but marked difference was observed between men receiving the same dose. For example, over a period of 48 weeks the highest rate measured for one man was 0.11 mg./hr. while the lowest rate for another in the same group was 0.15 mg./hr. Their mean rates during this period were 0.081 and 0.270 mg./hr., respectively. The difference was highly significant ($P < 0.001$).

DDT was lost from storage in fat slowly after dosing was stopped. The concentration remaining following 25.5 months of recovery was from 32 percent to 36 percent of the maximum stored for those who had received 35 mg./man/day but was 66 percent for those who received only 3.5 mg./man/day, indicating slower loss at lower storage levels.

Recently, DDT has been used on an experimental basis at dosage rates varying from 0.3 to 3 mg./kg./day for periods up to 7 months in an attempt to decrease serum bilirubin levels in selected jaundiced patients. No side effects were observed. No improvement was noted in patients with jaundice based on cirrhosis who had no demonstrated liver enzymes deficiency. However, in a patient with familial, nonhemolytic, unconjugated jaundice based on a deficiency of glucuronyltransferase, treatment with DDT rapidly reduced the plasma bilirubin level to the normal range and relieved the patient of nausea and malaise from which he had suffered intermittently. The liver function tests as well as other laboratory findings remained normal. The improvement was maintained during the 6 months when DDT was administered, and had persisted for 7 additional months at the time the report was written. In this case, a dosage of 1.5 mg./kg./day produced a steady rise in plasma levels of p,p′–DDT from an initial level of 0.005 p.p.m. to a maximum of 1.33 p.p.m. at the end of treatment. At this time, the concentration in body fat was 203 p.p.m. Plasma levels fell slowly after dosing was stopped (Thompson *et al.*, 1969). The highest daily intake in this series was six times greater than the highest level administered in earlier studies and about 7,500 times greater than the DDT intake of the general population. The highest value for p,p′–DDT in serum observed in the entire series was 1.330 p.p.m. compared to 0.996 p.p.m., the highest value reported by Laws *et al.* (1967) for formulating plant workers.

Experimental oral exposure to DDD.—DDD is an insecticide in its own right and a metabolite of DDT. An attempt has been made to use the compound as a drug to control different forms of adrenal overproduction of corticoids in man. The attempt was originally based on the demonstration that DDD (Nelson and Woodard, 1948; 1949), especially o,p′–DDD (Cueto and Brown, 1962) causes gross atrophy of the adrenals and degeneration of the cells of its inner cortex in dogs. This essentially experimental use has met with limited success. The dosage given has varied from 7 to 285 mg./kg./day, but a dosage of approximately 100 mg./kg./day for many weeks has been necessary to produce any benefit in man (Bergenstal *et al.*, 1960; Bledsoe *et al.*, 1964; Gallagher *et al.*, 1962; Southern *et al.*, 1966a, 1966b; Verdon *et al.*, 1962; and Wallace *et al.*, 1961). In contrast, a rate of only 4 mg./kg./day is required to produce marked atrophy of the adrenal in the dog. Kupfer (1967) reviewed the extensive literature indicating that the effect in man and other species except the dog is caused by stimulation of corticoid metabolism by massive doses of o,p′–DDD and not to any direct effect on the adrenal. Southern *et al.* (1966a, 1966b) agreed that the effect was predominantly extra-adrenal in man when the drug was first given but offered evidence that adrenal secretion of cortisol was eventually reduced.

Even large doses of o,p′–DDD cause no histological alteration of the adrenals in man (Wallace *et al.*, 1961) Doses in the therapeutic range, (specifically those between 110 and 140 mg./kg./day) produced no detectable injury to the liver, kidney, or bone marrow. All patients treated in this way experienced significant anorexia and nausea, and some showed central nervous system depression varying from lethargy to somnolence. These toxic effects cleared when dosing was discontinued (Bergentstal *et al.*, 1960).

Experimental oral exposure to methoxychlor.—Men were fed methoxychlor at dosage levels of 0, 0.5, 1.0, and 2.0 mg./kg./day for 8 weeks. Careful clinical observations were made, and repeated samples were taken for hematology, biochemistry, and urinalysis. At the end of the study, biopsies of fat, testis, bone marrow, liver, and small intestine were obtained for multiple basic studies including biochemistry, electron microscopy, and gas-liquid chromatic lipid analyses. The studies indicated the safety of methoxychlor at 200 times the maximum permissible limit which is far greater than the actual intake of methoxychlor by people in the general population (Stein *et al.*, 1965).

Experimental oral exposure to dieldrin.—In order to determine the relationships between absorption and accumulation of dieldrin in man, a pharmacodynamic study was carried out with four groups of volunteers (3 to 4 people per group) who were given daily dosages of the active ingredient in gelatin capsules for 2 years (Hunter and Robinson, 1967; Hunter *et al.*, 1969). The dosage levels were 0, 0.01, 0.05, and 0.211 mg./man/day. These intentional doses were in addition to an estimated daily intake of 0.014 mg. from food. In view of the weight of the men involved, the highest total intake was in the range of 0.0028 to 0.0036 mg./kg./day. Dieldrin concentrations in the blood and adipose tissues, urinalysis, EEG studies, polygraphic recording of cardiorespiratory function, electromyographic studies, blood chemistry, estimation of blood plasma protein and urea, activity of plasma alkaline phosphatase, SGPT, SGOT, and the activity of erythrocyte and plasma cholinesterase were determined on the volunteers. Full clinical examinations were made during the 3d, 9th, 15th, 18th, and 24th months of exposure.

The subjects selected were 13 healthy men without a history of recent occupational exposure to pesticides and whose ages ranged from 21 to 52 years.

The results of all ancillary observations showed no differences between the exposed and control subjects.

The only changes found in the exposed subjects were increases in the concentration of dieldrin in the samples of adipose tissue and blood. A relationship was established between concentrations of the compound in whole blood or adipose tissue, and the daily dose and duration of exposure. This relationship is of a curvilinear type with a finite upper limit (asymptote).

The upper limits of storage were 0.0202 p.p.m. in blood and 2.85 p.p.m. in adipose tissue of the men given 0.211 mg./man/day.

The concentration of dieldrin in adipose tissue was related to that in whole blood, the ratio of the concentrations being 136 (confidence limits, $p=0.95$, 109–170).

An estimate of the half-life of dieldrin in the blood of man, based on the decrease in tissue levels during the first 9 months post-exposure period was 369 days.

In summary, this study demonstrated three major facts:

First, the lack of any effect of the ingestion of amounts of dieldrin up to 0.225 mg./man/day over a period of 2 years on the health of male volunteers and on the results of a very extensive battery of laboratory tests, including the measurements of hepatic and nervous system function.

Second, the development of a steady state of storage associated with the continued intake of specified quantities of dieldrin.

Third, the existence of a dependable relation between the level of intake and of storage in blood and adipose tissue. This relation allows the calculation of levels of intake or absorption by the simple determination of dieldrin levels in blood, or in fat of people at the steady state of storage.

The dynamics of storage in man has been explored more thoroughly from a mathematical standpoint for dieldrin than for DDT. However, it is clear that the general pattern is the same for both. It is a pattern well established in pharmacology and it fully explains the steady state of storage corresponding to each level of intake. The experimental results, in combination with an understanding of the principles involved, offers assurance there will be no increase in storage in different population groups in the absence of increased exposure.

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Epidemiology of pesticides

Man, today, is in a dilemma as to how to further proceed with his chemical control of pests. On the one hand, the very obvious benefits that his society has attained by past pest control practices have vastly improved his comfort and standard of living. Now in many areas of the world he is no longer bothered by vector borne infectious diseases; in many countries his improved food and fiber technology resulting from pesticide usage has somewhat simplified his problems of hunger and starvation. On the other hand, the visible costs of this environmental manipulation are more obvious. The ecological signals from fish and birds are of concern. Even the health implications of his own residues for himself and his progeny are still not satisfactorily answered. Reassurances based upon the reports of the health and well being of pesticide workers or information on animal experimentations have not been sufficient to totally remove these doubts. Animal studies on carcinogenesis, mutagenesis, and the demonstration of enzyme induction are all events which have caused him to reappraise his future policies. Naturally, therefore, the continued use of certain persistent pesticides comes under review. Herein lies the crux of his dilemma. If specific restrictions are introduced, what will be the consequences of shifting to shorter acting pesticides? Will there be greater incidence of acute pesticide poisonings since for the most part toxicity, especially dermal is greater? What effects will this produce on the price of food and the cost of living? What will be the repercussions of changes on the developing countries, where the need for increased food production and malaria control is paramount? Although such questions can only be totally answered by synthesizing the recommendation of many disciplines, epidemiology is one which can provide a significant contribution. This section of the report, reviewing the evidence from the more recent and pertinent literature assesses the health hazards from an epidemiologic point of view, measuring the health effects consequent upon both acute and chronic pesticide exposure.

The epidemiology of acute pesticide poisonings.—Before reviewing the agent host attributes of acute pesticide poisonings, some information on the incidence data is important. Poisonings by pesticides is not in most areas a notifiable disease, so that the acquisition of true incidence is deficient in the more developed countries and practically impossible in developing countries.

In the United States, the mortality rate has been given as 1 per 1 million and the ratio of fatal and nonfatal poisonings expressed as 1 to 13 in one study and 1 to 75 in another study depending upon the criteria of severity (Hayes, W. J., 1964). The death rates associated with accidental poisoning by solids and liquids have remained relatively stable since 1939. Between 1945 and 1959 there was a decrease in the death rate associated with accidental poisoning by gases and vapors. Since 1939 the death rates associated with solids and liquids, and gases and vapors have been about equal totaling 2 per 100,000 population for all types. Pesticides contribute a substantial proportion of cases caused by all solids and liquids, a proportion which has ranged from 12.8 percent and 6 percent in different years (McCarthy, M. A., 1967). A physician and a medical examiner reviewed in 1961 assessed that 90 percent of poisons due to pesticides were correctly diagnosed (Hayes, W. J.; Pirkle, C. I., 1966). In spite of this however, it has been found that many deaths ultimately shown to be due to pesticides were not correctly diagnosed until they came to autopsy. Thus, of eight cases of organophosphate insecticide poisonings occuring in Dade County in 1963, six were totally unsuspected until coming to autopsy and chemical analysis (Davis, J. H., 1963). In a review of 1,000 deaths subjected to medico-legal death investigation over a 10-year period 9.7 percent were due to pesticides and in the under 5-year-age group pesticides were the leading cause of death (49 percent) far exceeded deaths due to all medications including aspirin (24 percent) (Davis, J. H., 1967). Parathion was the leading cause in this series and the indiscriminate use of this chemical in the home and the ready availability of the discarded pesticide containers were significant contributory factors (Plates A and B).

Another variable that has to be considered is reflected in the availability of medical facilities for death investigation. Between one-half and two-thirds of the approximate 3,000 counties of the United States have little or no professional facilities for death investigations (Davis, J. H. et al., 1969). In Puerto Rico, pesticides are the leading cause of fatal poisonings (Kaye, S., 1967). The limitation of present incidence data based upon nationwide mortality data has been described (Hayes, W. J., 1964; Hayes, W. J.; Pirkle, C. I., 1966; Reich, G. A. et al., 1968). A special survey of death due to solids and liquids was conducted in 1964 (Reich, G. A. et al.[a], 1968). Eighteen hundred and seventeen deaths were so categorized. Two hundred and eighty-three of these were assigned to category E888 (W.H.O., Geneva, 1957). Ninety of these were due to pesticides and more than one-third of the deaths occurred in preschool children. In Dade County, both fatal and nonfatal poisonings by pesticides fell into one of three distinct groups: (a) Young children who accidently ingested pesticides, (b) young to middle age adult males who are occupationally exposed, and (c) middle age to older adults who suicidally ingest pesticides (Reich, G. A. et al.[a], 1968; Davies, J. E. et al., 1967). The case fatality rates in these three groups was 22 percent, 4 percent, and 70 percent; and the percentage due to organophosphates was 72 percent, 96 percent, and 60 percent. Parathion was by far the most significant organophosphate insecticide.

This century has seen marked improvement in the safe handling of these chemicals even though the extent of such use has increased greatly. Testimony to this is the reduced case fatality rate in the occupationally exposed group mentioned in the previous page.

In the countries where there are less government controls and the inherent problems of toxicity are ill understood, incident data is usually provided by reports of epidemics of pesticide poisonings. Some of these are listed in Table I (Anderson, L. S. et al., 1965; Cam, C., Nigogosyan, 1963, Coble, Y. et al., 1967; Davies, G. M., Lewis, I., 1956; Gomez Ulloa, M. et al., 1967–68; Haq, I. U., 1963; Jalili, M. A., Abbasi, A. H., 1961; Kanagaratnam, K. et al., 1960; Karunakaran, C. O., 1958, Lange, P. F., Terveer, J., 1954; Lemmon, A. B., 1956; Marquez Mayaudon, E. et al., 1968; McGee, L. C. et al., 1966; Quinby, G. E., Lemmon, A. B., 1958; Milby, T. H. et al., 1964; Ol Achrafi, T., 1963; Ordonez, J. V. et al., 1966, Przyborowski, T. et al., 1962; Schmid, R., 1960, Warren, M. C. et al., 1963; Weeks, D. E., 1967; Wishahi, A. et al., 1958; West, I., 1965; Armstrong, R. W. et al., 1969; Hatcher & Wiseman, 1969).

Morbidity data can best be extrapolated from areas where reporting occupational poisoning by pesticides is mandatory. In California such reports are required if work injury involves the approximately 80 percent of employed persons in that State covered by the California's Workmen's Compensation Law (West, I., Milby, T. H., 1965). Between 1960 and 1963 the number of reports of work injury from pesticides has ranged from 827 to 1,013 annually. The number of persons at risk is estimated to be approximately 250,000 (California Department of Public Health, 1960, 1961, 1962, 1963). About one half of these reports deal with skin disease and about one third with systemic illness. During this same period pesticides caused 21 deaths in California. Of the total, five were associated with occupational exposure while 16 other deaths, largely of children, were not occupational in origin (West, I., Milby, T. H., 1965).

Agent factors.—Table II lists the pesticide production 1960–66 in the United States. Seventy-nine different types of pesticides have been reported as causing human poisonings. Of these there were 19 different organophosphate compounds, 20 organochlorine compounds, five carbamates, eight herbicides, and miscellany of other materials. Figures I and II from A.P.H.A.'s manual "Safe Use of Pesticides, 1967) show the relative dermal and oral toxicities of the more commonly used organophosphates and organochlorines. Understandably agent contribution to the epidemiology of acute pesticide intoxication is largely a reflection of toxicity and availability. It has been shown that insofar as occupational exposure is concerned, the dermal LD_{50} is more indicative of what can be expected from poisoning in people; thus parathion causes more poisoning than methyl-parathion, a feature which might be explained on the basis of the greater dermal toxicity in the former (Hayes, W. J., 1964).

In the United States, parathion and phosdrin, are the organophosphates which have caused the most number of pesticide poisonings. In Dade County, Florida, 46 percent of 72 pesticide fatalities between 1959 and 1965 were due to parathion (Davies, J. E. et al., 1967). In south Texas, in a study of 129 pesticide poisonings, 70 of which occurred in 1964, 98 percent were caused by ethyl or methyl parathion and the route of exposure was dermal in 98 percent of these (Reich. G. A. et al., 1968[6]).

The host.—In the United States, epidemiologically two population groups are recognized in pesticide poisonings—children and adults. The essential demographic characteristics are figuratively shown in the attached description of 145 organophosphate poisonings occurring in south Florida (See Figure 3) (Davies, J.E., et al., 1969). In this area, the child problem can almost entirely be equated with the accidental ingestion of parathion by a 2½ year old Negro male infant. Recently with legislation prohibiting the use of this insecticide, in an urban city, the position has improved considerably. The problem of accidental poisoning by a child with insecticides is really a totally preventable problem. With education and legislation this problem could end (and is) being improved. An extensive nationwide program is currently operational advising parents of the dangers of these economic poisonings and advocating storing them out of reach of children and instructing them on the careful and safe disposal of containers with added warnings of the hazards of putting pesticides in other bottles or containers, such as pop bottles or nursing bottles.

Insofar as adult poisonings are concerned, if suicide or homicide are excluded, the problem is being met by an extensive agricultural educational program. Education, surveillance, improved safety technology in the production levels of pesticides and in the removal and destruction of air and water effluent are also contributing factors together with the use and changing of protective clothing and the favoring of pesticides of an intermediate human toxicity.

In the developing countries the problem seems to be due to gaps in storage and transportation and ease which used containers are subsequently acquired and used domestically for storage of food. Legislation toward the separate handling of pesticides and food in transportation (sea as well as on land) would lessen the occurrence of epidemics of poisonings. The domestic use of the used container is a common source of food contamination in these situations. A universal policy on container manufacture, storage, and disposal is urgently needed and would benefit the so called "developed" and developing countries alike. In the former areas, warning notices of toxicity and use should be spelled out in the language of the particular country.

In the United States, a special area of concern has stemmed from the availability of chemicals for roach control. Thalium was an example of a highly toxic material which was far too dangerous to be used in the home and it is only within the last 2 years and after several hundred poisonings that this material has been removed from the market (Reed, D. et al., 1963). The same situation existed with white phosphorous paste, which has been responsible for 13 deaths in Dade County between 1956 and 1968, and only recently has this material been withdrawn from local stores; the use of materials such as these for roach and rodent control are surely examples of over kill.

Nationwide statistics on fatal and nonfatal poisonings are hard to come by, so that those agencies who carry the burden of responsibility of pesticide approval and labeling are not able to obtain a true picture of the acute toxicity effects in the nation. Only by means of a comprehensive pesticide monitoring network can the total health effects of acute intoxication be truly assessed.

TABLE I.—*Epidemics of poisoning by pesticides*

Kind of accident	Pesticide involved	Material contamination	Number of cases	Number of death	Location	Reference
Spillage during transport or storage.	Endrin	Flour	159	0	Wales	Davies & Lewis, 1956.
	Endrin	Flour	3	0	Egypt	Coble *et al.*, 1967.
	Endrin	Flour	691	24	Qatar	Weeks *et al.*, 1967.
	Endrin	Flour	183	2	S. Arabia	Weeks *et. al.*, 1967.
	Dieldrin	Food	21	0	Shipboard	Przyborowski *et al.*, 1962.
	Diazinon	Doughnut mix	20	0	U.S.A.	West, 1965.
	Parathion	Wheat	360	102	India	Karunakaran *et al.*, 1958.
	Parathion	Barley	38	9	Malaya	Kanagaratnam *et al.*, 1960.
	Parathion	Flour	200	8	Egypt	Wishahi *et al.*, 1958.
	Parathion	Flour	600	88	Colombia	Gomez Ulman *et al.*, 1967.
	Parathion	Sugar	300	17	Mexico	Marquez Mayaudon *et al.*, 1969.
	Parathion	Sheets	3	0	Canada	Anderson *et al.*, 1965.
	Mevinphos	Pants	6	0	U.S.A.	Warren *et al.*, 1963.

Eating formulation	Hexachlorobenzene	Seed-grain	>3, 000	[a] 3–11%	Turkey	Cam & Nigogosyan, 1963; Schmid, 1960.
	Organic mercury	Seed-grain	34	4	West Pakistan	Haq, 1963.
	Organic mercury	Seed-grain	321	35	Iraq	Jalili & Abbasi, 1961.
	Organic mercury	Seed-grain	45	20	Guatamala	Ordonez *et al.*, 1966.
	Warfarin	Bait	14	2	Korea	Lange & Terveer, 1954.
Improper application	Toxaphene	Collards	4	0	U.S.A	McGee *et al.*, 1952.
	Toxaphene	Chard	3	0	U.S.A	McGee *et al.*, 1952.
	Nicotine	Mustard	11	0	U.S.A	Lemmon, 1956.
	Parathion [b]		>17	15	Iran	Ol Achrafi, 1963.
Miscellaneous	Parathion	Crops	>400	0	U.S.A	Quinby & Lemmon, 1958, Milby *et al.*, 1964.
	Pentachlorophenol	Nursery linens	20	2	U.S.A	Armstrong *et al.*, 1969.
	Parathion	Crops	23	0	U.S.A	Hatcher & Wiseman, 1969.

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[a] 3 to 11% annually in different years. [b] Used as a treatment for lice.

Figure 1.—*Acute oral and dermal toxicity values for some phosphate ester pesticides.*

(Prepared by the Bureau of Occupational Health, State of California Department of Public Health. Copied with permission.)

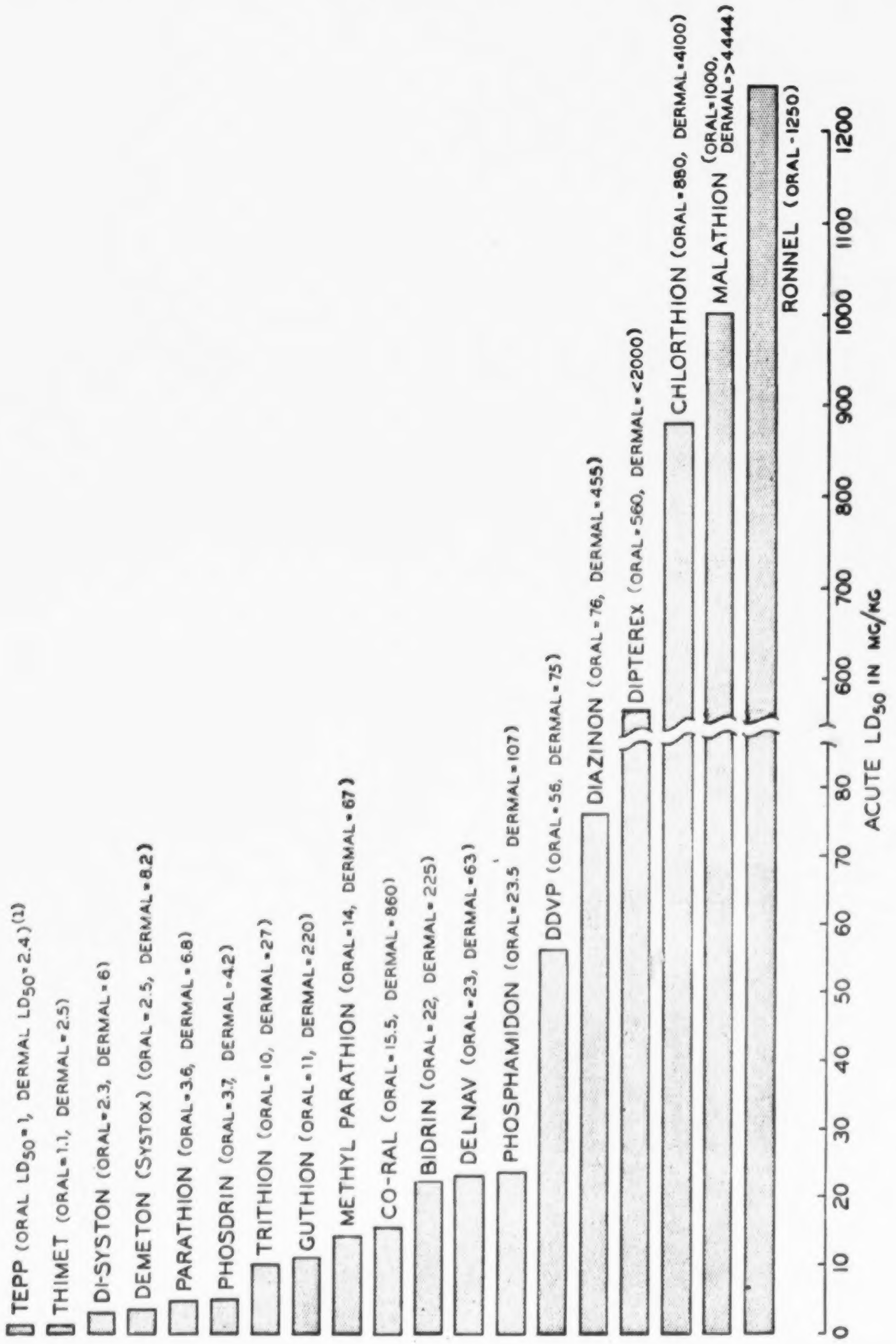


Figure 2.—*Acute oral and dermal toxicity values for some chlorinated hydrocarbon pesticides.*

(Prepared by the Bureau of Occupational Health, State of California Department of Public Health. Copied with permission.)

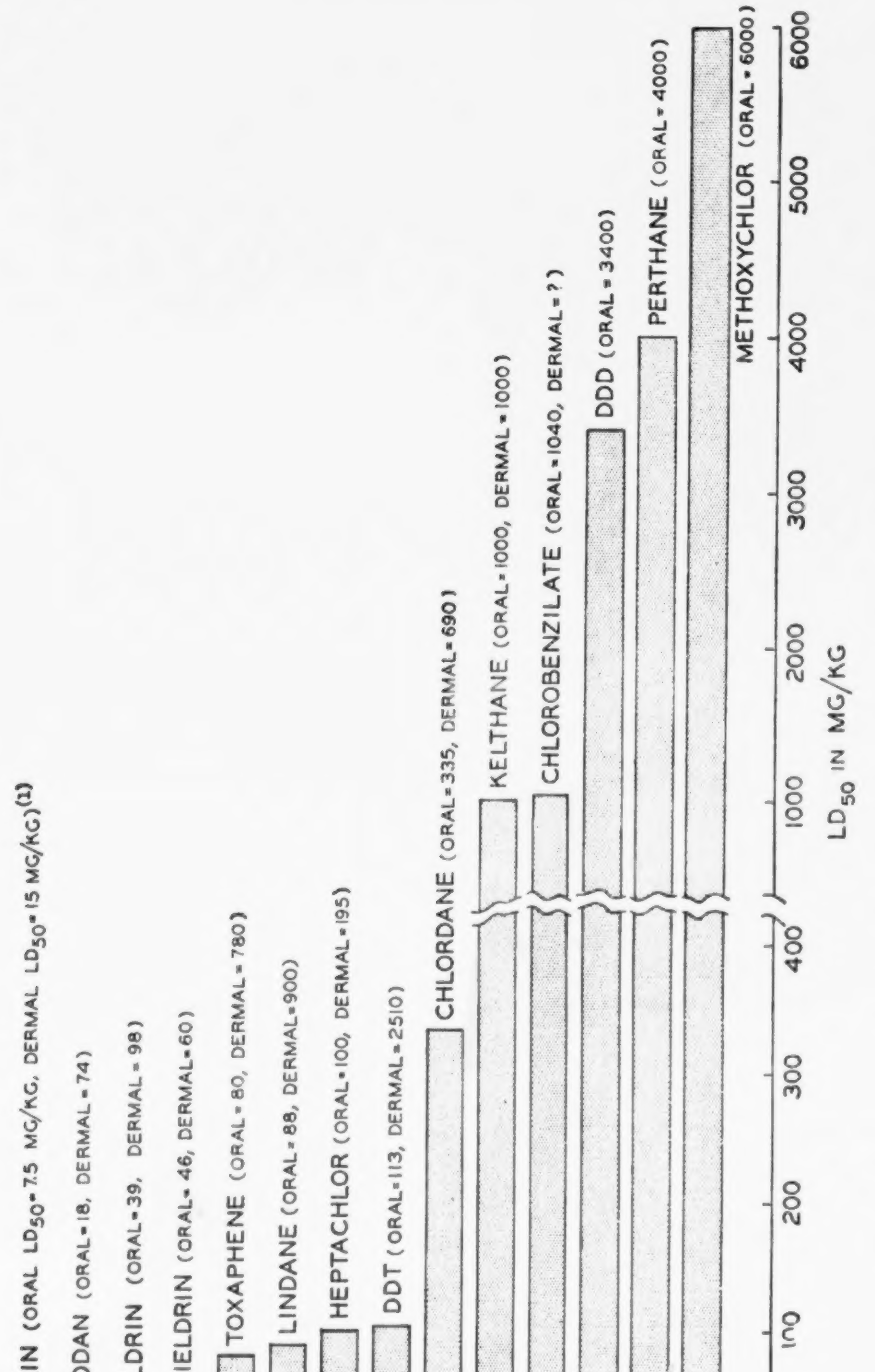
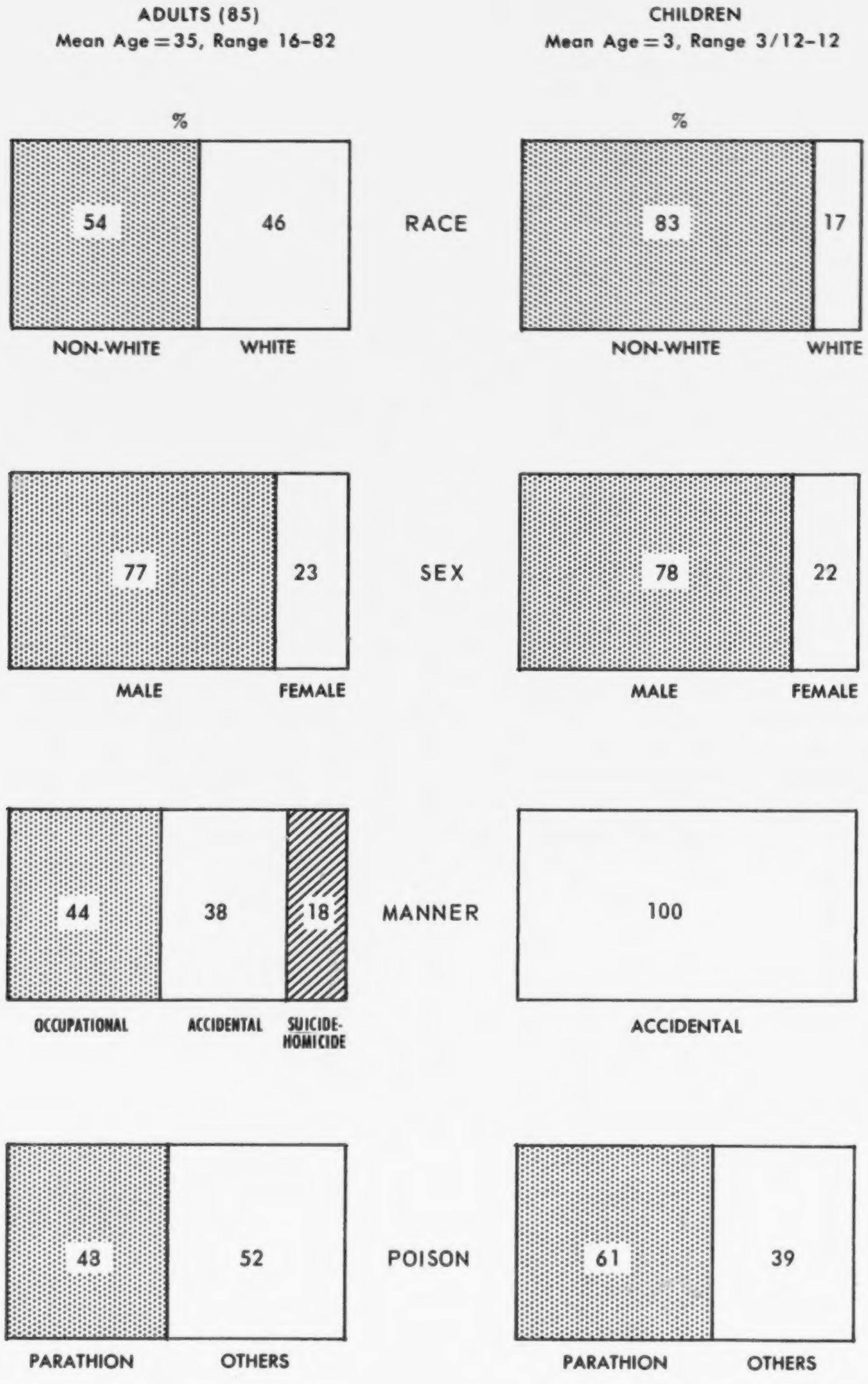


FIGURE 3.—*Epidemiology of pesticide poisoning cases in Dade County, Fla., 1963–1968.*



SOURCE: Davies, J. E. et al., 1969 (Reference No. 6)

TABLE II.—*U.S. pesticide production annual production*

[Thousand pounds]

Compound	1960	1961	1962	1963	1964	1965	1966
Calcium arsenate	6, 590	7, 944	4, 660	3, 310	6, 958	4, 192	2, 890
Lead arsenate	10, 062	10, 446	9, 990	7, 842	9, 258	7, 098	7, 328
Copper sulfate	116, 000	97, 168	79, 968	83, 272	83, 768	94, 656	103, 416
Aldrin-toxaphene group [1]	99, 671	103, 763	106, 276	105, 986	105, 296	118, 832	130, 470
Benzene hexachloride [2]	37, 444	25, 080	12, 022	6, 778	------	------	------
DDT	164, 180	171, 438	167, 032	178, 913	123, 709	140, 783	141, 349
Methyl bromide	12, 659	12, 892	12, 757	17, 394	16, 994	14, 303	16, 345
Methyl parathion	11, 794	18, 527	16, 156	15, 999	18, 640	29, 111	35, 862
Parathion	7, 434	8, 423	8, 786	------	12, 768	16, 607	19, 444
Ferbam	2, 529	3, 091	2, 966	2, 500	1, 838	2, 384	1, 379
Nabam	2, 978	3, 675	4, 216	2, 420	2, 251	2, 489	2, 053
Zineb	------	8, 313	------	3, 755	6, 664	5, 075	4, 721
2,4-D acid	36, 185	43, 392	42, 997	46, 312	53, 714	63, 320	68, 182
2,4,5-T acid	6, 337	6, 909	8, 369	9, 090	11, 434	11, 601	15, 489
Other organic pesticides [3]	277, 229	305, 600	348, 967	380, 021	437, 043	477, 148	577, 816

[1] Includes the chlorinated compounds aldrin, chlordane, dieldrin, endrin, heptachlor, and toxaphene.

[2] Gross production (gamma isomer content was 7.7 million pounds in 1961, 3.4 million pounds in 1962, and 1.8 million pounds in 1963): no data since 1963.

[3] Includes such materials as dithiocarbamate fungicides, malathion, methoxychlor, captan, TDE, organic rodenticides, etc.: does not include some fumigants.

Source; U.S. Dept. of Agriculture, Agricultural Statistics 1968.

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Sunniland
NON-SELECTIVE WEED KILLER
DUSTLESS PARATHION 15 W
Trithion 2
ORTHO
TRIOX
Holiday
Black Leaf
ORTHO
LAWN DISEASE CONTROL
DOUBLE SPRINGS

PLATE A
(By permission and courtesy of Dr. J. H. Davis, Med. Ex. Dade County)

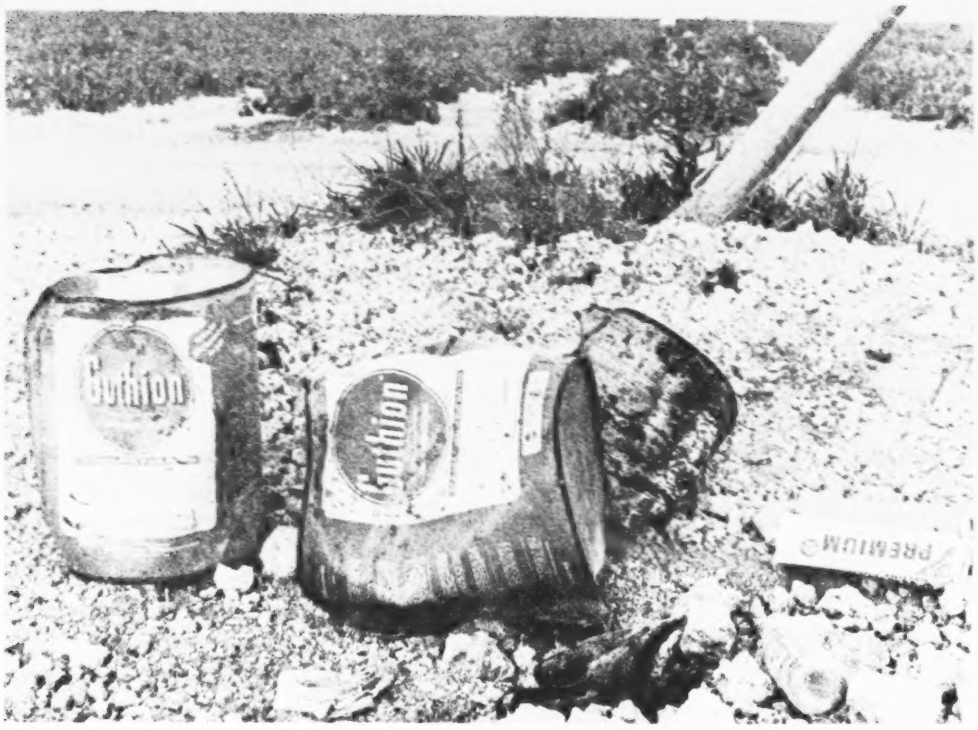

Plate B

(By permission and courtesy of Dr. J. H. Davis, Med. Ex. Dade County)

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Epidemiologic studies of the effects of pesticides on the general population.—Apart from the occurrence of acute poisoning, the only long term effect which can be unequivocally attributed to sustained exposure to organochlorine pesticides is the acquisition of a tissue residue. No casual association of these levels with disease has as yet been demonstrated, their magnitude being used largely as an epidemiologic tool reflective of national, geographic or secular doses of pesticides.

In the past epidemiological studies of the effects of pesticides on the general population using these tissue residues as biological indices of exposure, have either sought to demonstrate their association with health or disease, or they have compared differences in health experience associated with those variables of person, place and time which were uniquely reflective of the differentials of human pesticide exposure (e.g. occupational exposure, rural versus urban exposures or exposure in the pre and post DDT eras).

Human pesticide residues. A changing profile, introduction.—Although qualitative and quantitative pitfalls in pesticide analytical chemistry still persist in certain areas, the improvements of methodology and instrumentation have reached a degree that permits meaningful epidemiological interpretation of the human pesticide prevalance (Robinson, J., 1969). The last decade has seen a substantial growth of information on both the qualitative and quantitative nature of the pesticide spectrum in man. Thus, qualitatively, different surveys have demonstrated traces of the *isomers of DDT and its metabolites* (Hayes, 1958; Laug et al., 1958; Hayes et al., 1956; Hayes et al., 1958; Quinby et al., 1965; Dale and Quinby, 1963; Hoffman et al., 1964; Zavon et al., 1956; Hayes et al., 1965; Schafer, M. L. and Campbell, J. E., 1966; Radomski et al., 1968; Davies et al., 1968; Fiserova-Bergerova, V. et al., 1967; Davies et al., 1965; Hoffman et al., 1967; Casarett, L. J. et al., 1968; Read and McKinley, 1961; Maier-Bode, 1960; Hunter et al., 1963; Egan et al., 1965; Robinson et al., 1965; Hayes et al., 1963; Denes, 1962; Dale et al., 1965; Wasserman et al., 1965; Brown, J. R., 1967; Engst, R. et al., 1967; Robinson, J. and Hunter, C. G. 1966; Cassidy, W. et al., 1967; Abbott, D. C. et al., 1968; Kanitz

S. and Castello, G., 1966; Del Vecchio, V. and Leoni, V., 1967; Paccagnella, B. et al., 1967; Bick, M., 1967; Wasserman, M. et al., 1968; Maes, R. and Heyndrickx, A., 1966; Halacka, K. et al., 1965; Weihe, M., 1966; de Vlieger, M. et al., 1968; Wasserman, M. et al., 1967; Brewerton, H. V. and McGrath, H. J. W., 1967; Bronisz, H. et al., 1967; Llinares, V. M. and Wasserman, M., 1968), *BHC and its isomers, dieldrin, heptachlor epoxide in various population surveys* (Dale and Quinby, 1963; Hoffman et al., 1964; Zavon et al., 1965; Hayes et al., 1965; Hunter et al., 1963; Egan et al., 1965; Robinson et al., 1965; Hayes et al., 1963; Dale et al., 1965; Schafer, M. L. and Campbell, J. E., 1966; Fiserova-Bergerova, V. et al., 1967; Hoffman, W. S. et al., 1968; Edmundson, W. F. et al., 1968; Radomski, J. L. et al., 1968; Casarett, L. J. et al., 1968; Robinson, J. and Hunter, C. G., 1966; Abbott, D. C. et al., 1968; Cassidy, W. et al., 1967; Bick, M., 1967; Wasserman, M. et al., 1968; Kanitz, S. and Castello, G., 1966; Del Vecchio, V. and Leoni, V., 1967; Paccagnella, B. et al., 1967; Brown, J. R. 1967; de Vlieger, M. et al., 1968; Maes, R. and Heyndrickx, A., 1966; Denes, A., 1966; Brewerton, H. V. and McGrath, H. J. W., 1967; Engst, R. et al., 1967). At this time, residues of polychlorinated biphenyl's have not complicated pesticide residue interpretation in human adipose surveys. In addition to adipose surveys concentration of pesticide levels in blood have recently been studied (Dale, W. E. et al., 1966; Robinson, J., 1963; Schmit, J. A. et al., 1964; Kadis, V. W. and Jonasson, O. J., 1965; Schaefer, M. L. and Campbell, J. E., 1966; Brown V. K. H. et al., 1964; Robinson, J. and Hunter, C. G., 1966; Davies, J. E. et al. 1969[a]; Nachman, G. A. et al., 1969).

Quantitatively, greatest emphasis has been placed on the prevalence of DDT and its metabolites, dieldrin and BHC; considerable inferences have been placed on secular and geographical interpretation of these levels; thus data were used to express the magnitudes of human contamination in different countries and were quoted as indices of pesticide intake of the country. In addition, comparisons between countries were made, and when collected at different periods of time were used as indices of secular change (Hoffman et al., 1964; Quinby et al., 1965; Hoffman et al., 1967; Brown, J. R., 1967; Hoffman, W. S., 1968). Several authorities claimed the United States exposure to certain persistent pesticides were going down based on data from samples collected from different years. Smaller residue studies by comparing levels in meat abstainers and meat eaters sought to identify the special contributions of certain special food items such as meat (Hayes, W. J. Jr., 1958).

More recently a new interpretative function of pesticide residue levels can be seen in the literature. Levels have been described in specific diseases; some have demonstrated an association of pathol-

ogies with high residue value of DDT and its metabolites (Radomski, J. L. et al., 1968; Cassarett, L. J. et al., 1968), whereas others have found no association with a wide variety of pathologies (Robinson, J. et al., 1965; Hoffman, W. S. et al., 1967). This new and strictly epidemiological use of pesticide residue highlights the urgent need for an understanding of the significance of pesticide tissue residues in the normal individual before interpretations can be made of levels in the sick.

The epidemiology of adipose and blood residues of DDT and DDE in humans—Interpretation of the "level".—In the more recent population surveys of pesticide residues, two methodological improvements have contributed much to the better understanding of human pesticide residues. The first of these was the presentation of adipose data of DDT and its metabolites and dieldrin from larger and more stratified samples of populations (Wasserman, M., et al., 1967; Davies, J. E., et al., 1968; Edmundson, W. F., et al., 1968; Davies, J. E., et al., 1969[a]; Yobs, A., 1969). All over the world meaningful understanding of human pesticide residues has been severely restricted by the smallness of sample size and by the absence of stratification. Thus, the largest survey from an area of the United States was provided by data from 944 adipose samples collected by Hoffman, from Chicago. In response to a request from the Secretary's Commission on Environmental Health Aspects of Pesticides, most useful, valuable, and unpublished material has been provided by the human monitoring program of the Division of Community Studies, OPS, FDA, Atlanta, Ga. (Dr. Ann Yobs). A more meaningful description of the qualitative character of the U.S. human pesticide residue profile is provided in table III for the fiscal year 1967. These are data from 734 samples (88 of whom were Negro) collected from 10 States of the Union. Table IV, presenting cumulative data from the United States of America for 1968, stratified by age and race, providing information on the prevalance of DDT and its metabolites and dieldrin from 4,696 adipose residues obtained from the human monitoring program.

The second event has been the development of methods for measurement of organochlorine pesticides in blood; blood levels for DDT-derived materials and dieldrin were found to correlate significantly with dipose levels (Robinson, J., 1969; Davies, J. E., et al., 1969[a]; Schaefer, M. L., and Campbell, J. E., 1966). Thus, a far more acceptable and readily obtainable tissue was made available, permitting larger sampling and greater stratification of various study groups. Review of these data, provided by blood and adipose surveys, suggest that whereas dieldrin levels are essentially extremely homogenous, there are significant contributions of person, place, and time to the epidemiology of DDT residues in man. These are discussed hereinafter.

Personal variables.—Besides the obvious contribution of occupation, significant differences of DDT and DDE, but not for dieldrin have been associated in fat and blood with sex, race, age, and socioeconomic variables.

Sex differences.—Insofar as DDT and its metabolites are concerned whereas some investigators have found a significant association of residues with sex, others have failed to find any differences, and others again have found in the Negro race only. Thus, positive association of DDT residues with males was demonstrated in adipose surveys by Wasserman, M., et al., 1965; Dale, W. E., et al., 1965; Robinson, J., et al., 1965; Laug, E. P. et al., 1951; Hayes, W. J., Jr., et al., 1958; Davies, J. E., et al., 1969[a]. HEOD or dieldrin residues showed no sex differences in the United States (Edmundson W. F., et al., 1968; Hoffman, W. S., et al., 1967), but in four surveys in the United Kingdom higher levels of dieldrin were observed in males than females (Robinson, J., et al., 1965; Hunter, C. G., et al., 1963; Egan, H., et al., 1965; Abbott, D. C. et al., 1968). The reason why some surveys have shown this association and others have not may be reflective of small sample size. In the largest survey from the United States of America provided by data from the human monitoring program, no differences due to sex were observed for dieldrin or DDT residues.

Ethnic differences.—In Israel no ethnic differences were observed in the comparison of levels of DDT-derived materials from adipose specimens obtained from Ashekenazim, Sephardim, native Israelis, Yemen, and Indian (Wasserman, M., et al., 1967). In contrast, in the United States, levels of DDT-derived materials were signficantly higher in nonwhites than in whites. This has been seen both in blood and fat from community pesticide studies in California and Florida, and from studies in Chicago (Hoffman, W. S., et al., 1967); in the data from the monitoring program both for the fiscal years of 1967 and 1968, residues of DDT were significantly higher in the Negro than in the white. In the Hawaiian community pesticide studies, residues of DDT were no higher in residents of Caucasian decent than in native-born Hawaiians or persons of Japanese decent. In contrast to DDT, no race differences were observed for dieldrin from 146 autopsies (44 Negro) from persons accidentally killed in Dade County in 1965–1967 (Edmundson, W. F., et al., 1968) though in Chicago, Negro males (23) had levels of BHC significantly higher than in whites (346) (Hoffman, W. S., et al., 1967). At the present time, the race-associated differences observed for DDT appears to be unequivocal and is confirmed by data from the human monitoring program for 1967 and 1968; race associated differences for the other persistent pesticides was not suggested by these data.

Age differences.—Most adipose surveys have not included a sizable number of pesticide residues from the younger groups so that information on the effect of age has been limited. Where surveys have been published presenting data from a significant number of younger persons, levels of DDT residues appeared to be lower in children under 10 years than in the older age groups. This is shown in table V from data from Israel (Wasserman, M., et al., 1967) and from south Florida (Davies, J. E., et al., 1968). In Israel the mean total DDT in fat from 71 children was 10.2 p.p.m. compared to 18.1 p.p.m for 133 persons in older age groups. In Florida the mean was 5.5 p.p.m. from white children under 5 years old as compared to 8.4 p.p.m. in the older age group, and 7.8 and 16.7 p.p.m. respectively for the Negro population. The same observation was noted from studies in Chicago (Hoffman) and by the community pesticide studies reports from Hawaii (Klemmer, H., Hawaiian community pesticide studies progress reports). This age effect was not seen for dieldrin (Edmundson, et al., 1968). The positive age association for DDT or DDE is very obvious for the United States of America from the nonwhite population in the data from 4,969 analyses shown in figure 3. When these data were broken down into Northern and Southern States the age effect for DDT residues was still striking for the Southern States but less obvious from the Northern States. Dieldrin data suggests no age association (fig. 4).

Socioeconomic differences.—Apart from the effects of occupation, information on the contribution of socioeconomic factors to human pesticide residues is not available. The association of greater fat and blood levels of DDT-derived materials with the Negro race in the United States is striking and begs for interpretation. The most plausible explanation would attribute this to socioeconomic factors and would incriminate such mechanisms as poor housing, inadequate garbage collection, deficient window screening, all furthering the multiplication of pests and the need for greater domestic use of insecticides. Reasonable though this hypothesis may seem, at this point in time it is still conjectural and it is probably too premature to conclude with any certainty, whether such differences are race associated or race dependent.

The effect of drugs.—Recently it has been shown that residues of DDT and its metabolites can be significantly lowered in persons taking phenobarbital and/or phenytoin (Davies, J. E., et al., 1969b). It is very probable that the future will see a growing body of knowledge on the potentiating and inhibiting effects of drugs on pesticide residues, and it is essential that the possible contribution of these drugs be assessed in all epidemiological studies wherein pesticide residue levels in disease States are being compared.

Place variable.—Since persons occupationally exposed to pesticides have higher values of DDT residues, it is not surprising that general population comparisons also show differences due to those attributes of place which are reflective of greater pesticide usage. In warmer climates, pests are more of a problem, and greater levels in tropical and subtropical climates would be anticipated. In Europe, residue data of DDT-derived materials were shown to be higher in the south and east (Robinson, J., 1969) and in the Middle East and Asia highest levels of DDT residues were seen in Israel (Wasserman, M., et al., 1965; Wasserman, M., et al., 1967) and in India (Dale, W. E., et al., 1965). Figures 5 and 6, depicting preliminary data of the prevalance of DDT-derived residues and dieldrin from 22 States in the continental United States, are presented on a race specific basis (Human Monitoring Data, fiscal year 1968). It will be seen that if States are grouped according to their mean monthly temperature, very obvious differences of DDT distributions associated with the mean monthly temperature become apparent. An arbitrary mean monthly temperature of 56° F. was taken for subdividing the States into low or cool temperatures and States with high or warm temperatures. In the data from the human monitoring program of adipose residues from 4,165 white persons, the mean total DDT for the cooler or low temperature States was 4.85 p.p.m. and for the warmer or higher States the mean was 9.21 p.p.m.; in contrast the dieldrin was 0.13 p.p.m. in both areas of the continent. The same place differences were noted for the Negroes. From adipose residues in 804 Negroes the total DDT was 7.86 p.p.m. in the cooler or lower temperature States and 14.37 p.p.m. in the warmer or high temperature States, whereas the dieldrin was 0.14 p.p.m. in the low or cooler States and 0.13 p.p.m. in the high or warmer States.

Time variable.—On an individual basis Hayes demonstrated that volunteers in various groups ingesting daily doses of DDT ranging from 0.0021 to 0.61 mg./kg./day reached a steady state of storage in fat in 18.8 to 21.5 months. Increased storage was only observed with increased exposure (Hayes, W. J., et al., 1956). Extrapolating from this study of individuals therefore to populations, we would expect pesticide residue levels to remain constant with age if the exposure did not change. Thus, age data on pesticide residues can provide information on societal equilibrium. If there is no increased exposure to DDT, then the various age groups should have the same mean DDT residues. If mean levels in the individual quinquenniums exhibit a stepladder effect then the concept of national equilibrium with regard to pesticide exposure must be questioned. As has been mentioned, age dependency of DDT residues has been observed in Israel, Chicago, Florida, and from the human monitoring data shown in figures 3 and 4. In the latter figure, increments of mean DDT residue values with each age group can be seen for both white and nonwhite up to the 21-to-25-year age group in the Southern States. Since DDT only became commercially available in 1948, the DDT age of our society today is approximately 21 years, we would expect that older age groups would all have the same average DDT residues. This is seen in the Chicago and Florida data and is suggested from the human monitoring data for the United States of America 1968. Why there is no leveling off in the nonwhite age group is not clear at this time, but since the numbers sampled in the older age group are very much larger than those in the younger age group this apparent steady increase may be an artifact due to the small numbers in the younger age groups. Whatever the explanation it is obvious that increased monitoring data in the future would clarify the secular changes of DDT tissue residues in our society.

Discussion.—In the light of these more recent data concerning the epidemiology of certain persistent pesticides, the data should be used to try to reappraise existing concepts and beliefs related to national levels, sources of pesticides time trends, and reported associations of certain diseases such as cancer, hypertension, and liver disease with high residues of DDT. It should be stated at the outset that the epidemiology of DDT and dieldrin appear to be very different, with striking associations of person, place, and time being noted insofar as human DDT residues are concerned but not seen with dieldrin residues. This finding sugests that for dieldrin it is relatively simple to speak of national levels and national time trends since large and stratified samples do not appear to be necessary, and furthermore the homogeneity of residues suport the concept that food is the main source of residues of this insecticide in man. The same cannot be said for DDT, and four epidemiological uses of DDT residue data can be reviewed and questioned in the light of these data on the distribution of DDT residues in our society.

1. National prevalence and international comparisons: Without demographic stratification and an appropriate adjustment for the contribution of location (including temperature) it does not seem correct, even today, to speak with any degree of confidence of the magnitude of national prevalence of DDT residues in man in the United States. Similarly international comparisons are really not very meaningful. Only when sufficient data becomes available from various national monitoring programs collecting tissues from meaningful strata of healthy populations, selected from areas which are reflective

of climatic and other environmental variables of this country, we will be able to describe the levels of DDT in man in the United States.

2. The role of dietary sources of persistent pesticides to the human body burden tissue load: Several investigators have concluded that food residues of DDT are of major importance as sources of human residues of this insecticide. Food is stated as contributing 89 percent of the total intake of DDT (Campbell, J. E. et al., 1965). Evidence supporting the major contribution of food is based upon feeding experiments. The marked heterogeneity of DDT residues of the various strata of our society together with the very obvious climatic differences observed in hot and cold areas of the world are strongly supportative of the significant contribution of nondietary sources of this pesticide to the human body burden. Consider the Human Monitoring Data. Mean values of DDT residues in fat were twice as high in the warmer Southern States than in the north. Negro levels, which were twice as high as whites, demonstrated the same geographical delineation. Theoretically, therefore, three possibilities come to mind:

(1) Southern persons eat different food than Northerners. This explanation would have to hold good for white and blacks alike and would have to explain areas outside of the United States.

(2) Food residues are higher in the south than in the north. This is unlikely because of the widespread interstate commerce and because of the existing food monitoring program.

(3) Food may be only partially contributive to the human body burden of DDT and nondietary sources of DDT may make up as much as 50 percent of the total body burden in warmer climates. Now what are the possible sources of this nondietary contributant? The amounts of DDT detected in air and water are present in very small concentrations. Air sampling techniques of pesticide residues are however still not entirely satisfactory and the available information on air and water sources of pesticide is very much less than what is known in the case of food.

Supportive of the nondietary contribution to body burden of DDT is the data from Alaska. Adipose residues from Alaskan eskimos for total DDT and DDE was 3.0 p.p.m. and 2.2 p.p.m. respectively (Durham, W. et al., 1961). The eskimos ate a predominately native diet shown to contain little or no DDT; the same type of information was observed in a study of nonambulatory institutionalized patients on a prolonged tube feeding gastrostomy diets whose diet was shown to be virtually free of DDT residues; adipose residues of total DDT-derived material and DDE were 1.47 p.p.m. and 1.28 p.p.m. respectively (Davies, J. E. et al., 1969^{b}).

In a comparison of DDE residues in blood in patients taking and not taking anticonvulsant drugs, nondrug taking controls, both white and Negro, more than 10 years old, fully ambulant and on normal diets and having similar values of DDE in blood to those observed in the normal population, exhibited the same race associated differences in spite of being institutionalized for many years. The persistence of this race associated difference under identical environmental conditions suggested acquisition of DDE levels prior to institutionalization. Clustering of DDE levels shown in table VI has been observed in families in Dade County and suggested that it was the home condition which contributed to the level. Thus, the role of the place variable reflected environmental exposure acquired early in life and from the home environment. To explore the nondietary source of DDT of the home environment, the authors carried out a small experiment using sentinel cats which were placed in homes of two families, representative of high and low blood residues. The sentinel cats were fed the same commercial pet food. The cat placed in the home characterized by high DDE levels, acquired a DDT blood level 10 times that observed of its litter mate placed in a family with low DDE levels. The explanation afforded was that the cats picked up soil and dust in the home on their fur, which they subsequently consumed in the process of cleaning themselves. The data from this study suggested that dust was a significant cotributant of nondietary sources of DDT in the home (Edmundson, W. F. et al., 1969).

Supportive of the dust concept were data provided by Aldrich, F. D. et al., 1967–68, in the Community Studies on Pesticides Project in Colorado (Progress Reports No. 11 and No. 15, 1967–68 respectively). Serum concentrations of DDT and DDE were studied in 70 families in 1966 and 1967 and were compared with values of this insecticide in soil and household dust collected from vacuum cleaner bags. Their data suggested that DDT and DDE concentrations in the home environment in random household samples were reflective of the serum values of the resident families. Household dust was used as a single index reflective of the pesticide input to the home, exclusive of dietary sources. Again in 1968, using 20 families, 12 of whom were high exposed (one member of the family being occupationally exposed to pesticides), the study was repeated and once again a strong association between levels of DDT and DDE in blood in the house dust was demonstrated. In addition, a strong association of blood levels of DDT and DDE in husband and wife of exposed families was demonstrated and concentrations were higher in the Colorado winter than summer. Collectively, therefore, these new data call for a reappraisal of the dominant role assigned to food as the major contributant of body burden residues of DDT. Data such as these make a strong case for the importance of pesticide research in the soil and dust as one of the sources of man's contamination of DDT. Whether such dust is contaminated by translocation of particulate DDT from remote sources, or from the overenthusiastic use of this insecticide in the home needs to be investigated.

3. Secular changes: Opinions have differed as to whether various national residue levels were on the increase, stationary or declining. In Canada, two surveys in 1949–50 and 1966 (Read, S. I., and McKinley, W. P., 1961; Brown, J. R., 1967) suggested levels were stationary and the same conclusion was reached in the United Kingdom 1963–64 (Abbott, D. C., et al., 1968) and 1965–66 in Israel (Wasserman, M. et al., 1965). In Great Britain levels of BHC, dieldrin and DDT were thought to have dropped and HEOD levels were lower in 1968 than in an earlier survey of the southeast of England; Quinby concludes that there was no change in levels in the United States between 1960 and 1961–62 (Quinby, G. E. et al., 1965) and Hoffman stated that levels of DDT-derived materials and BHC were lower in 1964–66 in Chicago than in 1962–63 (Hoffman, W. S. et al., 1967). Although significant race and sex differences were described no adjustment for these variables were made in reaching this conclusion. Qaife, M. L. et al., 1967, were the first to question the validity of these inferences and at the present it would appear that the question still remains not proven because of stratification weaknesses.

4. Pesticide levels and disease: Maier-Bode, H. (1961) was the first to explore the association of residues of DDT and DDE with diseases. He found no essential differences of DDT and DDE in 21 persons dying of cancer and 39 persons who died of other diseases. In a statistical survey, using the standard normal variation, and comparing the normal and abnormal tissues from 688 autopsies, Hoffman concluded that there was no significant correlation of levels of DDE, DDT, and of hexacychlorocyclohexane residues in fat with the presence or absence of abnormalities in the tissues (Hoffman, W. S. et al., 1967). However, although significant differences of DDT levels due to sex (approximately half of the samples were females) and race were observed, the authors made no provision for these variables in determining the significance of differences between pesticide residues in the normal and abnormal tissues. Others, such as Casarett, L. J. et al., 1968, and Radomski, J. L. et al., 1968 have found association of high levels of DDT-derived materials with several pathologies. In a study of 44 autopsies, Casarett, L. J. et al., 1968 noted that subjects with the highest total residues in tissues were those with evidence of emaciation, a

variety of cancers, and extensive focal or generalized pathologic conditions of the liver.

Their data suggest that these changes might be the consequence rather than the cause of the disease. Radomski, J. L. et al., 1968, in comparison of adipose and liver residues of DDT and its metabolites in healthy controls (persons accidentally or violently killed) and persons dying from diseases of the liver, the central nervous system and miscellaneous pathologies including several types of malignancies demonstrated: (1) A strong association of DDT residues with some central nervous system pathologies, carcinoma in different tissues, portal cirrhosis and hypertension and (2) an equally strong association of these pesticide residues in the sick with histories of the domestic use of insecticides. In discussing these results they were careful not to conclude that the disease association was causal but it was not clear whether the domestic use of insecticides were significantly higher in persons with terminal diseases than it was in healthy controls. In addition, they failed to stratify their data by race and since 69 percent of the control population were white, the differences observed could have been due to race associated differences if the disease population was largely Negro.

It is very probable that the future will see a steady increase of epidemiological studies investigating the prevalence of human pesticide residues in disease. Problems will arise as to whether evaluations are the consequence of the disease itself, or the drugs being used to treat the disease or whether they are the result of weight loss or the consequences of the body's inability to detoxify chemicals generally. All such possibilities will have to be discounted before causal roles can be inferred. DDT has recently been used to treat unconjugated bilirubinemia being more effective than phenobarbitone in promoting and sustaining enzyme induction. Enzyme induction and lowering of plasma bilirubin was first observed when plasma concentrations of DDT increased and was obviously active at concentrations of 200 p.p.b.; with continued DDT medication levels reached 1,300 p.p.m. (Thompson, R. P. H. et al., 1969). Plasma levels in excess of 200 p.p.b. are frequently observed under conditions of occupational exposure to DDT, so if one can extrapolate from the case, there may be significant liver microsomal enzyme induction under these circumstances. While this hepatic response is reflective of detoxifying mechanism, and is therefore, in one sense, an adaptive phenomena, the consequences may not always be beneficial. Because of enzyme induction the pharmacologic responses to a wide variety of drugs (and possibly steroids) may be significantly altered and much research in man is needed in this area as well as organochlorine surveillance in blood under conditions of extreme occupational exposure.

Besides being useful as an epidemiologic tool in studies of disease, the tissue residue is the single most useful expression of our total intake of persistent pesticides. Since food residue levels are reflective of only part of our pesticide intake, it is more necessary than ever to monitor our environmental health by studies of persistent pesticides from living tissues from man and from other biological sentinels in his environment. Only in this way can we measure the prevalence and secular changes of these persistent pesticides in our society.

TABLE III.—*Human monitoring survey—Laboratory findings of pesticide residue levels adipose tissue specimens* fiscal year 1967*

Laboratory		ppDDT	opDDT	ppDDD	ppDDE	opDDE	Total DDT Eq.	Pct. DDE	Hept. Epox.	Dieldrin	α-BHC	β-BHC	γ-BHC
A (n=457)	Max	7. 12	0. 41	1. 30	17. 42	0. 73	25. 47	100. 0	0. 44	1. 26	0. 20	1. 84	0. 48
	$\bar{X}$	0. 85	0. 01	0. 05	2. 88	0. 04	4. 17	79. 5	0. 02	0. 12	0. 00	0. 22	0. 01
	Med	0. 56	0. 00	0. 00	2. 34	0. 00	3. 32	80. 9	0. 00	0. 07	0. 00	0. 17	0. 00
	Min	0. 00	0. 00	0. 00	0. 00	0. 00	0. 00	0. 0	0. 00	0. 00	0. 00	0. 00	0. 00
B (n=89)	Max	18. 33	0. 91	1. 85	29. 13	2. 96	39. 06	100. 0	0. 40	0. 97	0. 00	8. 44	0. 00
	$\bar{X}$	2. 03	0. 03	0. 24	5. 78	0. 03	8. 80	71. 8	0. 09	0. 07	0. 00	0. 45	0. 00
	Med	1. 50	0. 00	0. 17	4. 35	0. 00	6. 60	75. 0	0. 07	0. 00	0. 00	0. 32	0. 00
	Min	0. 00	0. 00	0. 00	0. 06	0. 06	0. 07	16. 9	0. 00	0. 00	0. 00	0. 00	0. 00
C (n=164)	Max	27. 23	1. 54	4. 17	43. 43	0. 49	68. 34	95. 7	1. 56	2. 38	0. 19	1. 79	0. 72
	$\bar{X}$	2. 15	0. 20	0. 28	7. 01	0. 04	10. 54	73. 4	0. 11	0. 31	0. 02	0. 36	0. 03
	Med	1. 20	0. 13	0. 20	4. 86	0. 00	6. 94	75. 7	0. 09	0. 23	0. 00	0. 27	0. 00
	Min	0. 00	0. 00	0. 00	0. 00	0. 00	0. 00	0. 0	0. 00	0. 00	0. 00	0. 00	0. 00
D (n=24)	Max	6. 98	0. 17	0. 81	23. 77	0. 00	34. 07	100. 0	0. 16	0. 00	0. 30	0. 00	0. 00
	$\bar{X}$	1. 58	0. 02	0. 21	6. 92	0. 00	9. 54	79. 2	0. 02	0. 00	0. 05	0. 00	0. 00
	Med	1. 04	0. 00	0. 20	4. 41	0. 00	6. 92	76. 9	0. 00	0. 00	0. 00	0. 00	0. 00
	Min	0. 00	0. 00	0. 00	0. 13	0. 00	0. 14	47. 1	0. 00	0. 00	0. 00	0. 00	0. 00

*Adipose tissue residue levels given in parts per million (p.p.m.).

All values for opDDT were 0.00.

TABLE IV.—ADIPOSE RESIDUES UNITED STATES, HUMAN MONITORING DATA, fiscal year 1968; Pesticide Residue Levels in the General Population; Adipose Tissue (p.p.m.); Human Monitoring Survey, Division of Community Studies, OPS, FDA *(See Figure 3 following these tables.)*

Age	Sample			ppDDT		opDDT		ppDDE		Dieldrin		Total DDT[1]	
	W	NW		W	NW	W	NW	W	NW	W	NW	W	NW
0–5	72	31	Max	6.59	6.79	1.03	0.51	15.50	16.16	1.16	0.53	19.73	22.93
			Min	0.0	0.0	0.0	0.0	0.0	0.06	0.0	0.0	0.0	0.07
			$\bar{X}$	0.88	1.48	0.06	0.11	3.19	4.06	0.11	0.12	4.50	6.10
			S.D.	1.03	1.62	0.19	0.15	3.46	4.20	0.17	0.15	4.63	5.78
6–10	16	8	Max	1.46	5.60	4.69	0.56	35.12	12.69	0.71	0.47	40.41	16.91
			Min	0.0	0.29	0.0	0.0	0.0	0.35	0.0	0.0	0.67	0.72
			$\bar{X}$	0.78	2.22	0.52	0.11	3.85	4.79	0.14	0.12	5.59	7.66
			S.D.	0.49	1.99	1.18	0.21	8.45	4.23	0.21	0.17	9.48	5.31
11–15	30	8	Max	28.46	6.35	1.50	0.57	28.88	12.52	0.36	0.13	62.13	20.87
			Min	0.0	0.32	0.0	0.0	0.16	1.18	0.0	0.0	0.18	1.92
			$\bar{X}$	2.13	1.81	0.14	0.07	3.95	4.27	0.07	0.04	6.67	6.64
			S.D.	5.74	1.99	0.31	0.20	5.72	4.19	0.11	0.05	12.32	6.63
16–20	61	22	Max	14.44	10.79	0.55	0.47	38.20	35.11	0.32	0.29	57.49	50.37
			Min	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			$\bar{X}$	1.02	1.96	0.09	0.12	3.56	6.19	0.06	0.09	5.08	8.98
			S.D.	1.93	2.42	0.16	0.16	5.45	8.04	0.08	0.08	7.88	11.17

21–25	69	32	Max	90.00	7.12	0.86	0.81	12.63	23.44	0.53	0.33	90.48	31.38
			Min	0.0	0.34	0.0	0.0	0.0	0.89	0.0	0.0	0.0	1.54
			X̄	3.77	2.20	0.08	0.14	4.09	6.08	0.09	0.07	8.41	9.11
			S.D	15.04	1.75	0.17	0.19	3.48	4.90	0.11	0.10	15.06	6.70
26–30	100	18	Max	7.12	3.45	0.76	0.47	39.22	18.23	0.40	0.35	51.01	22.96
			Min	0.0	0.0	0.0	0.0	0.0	0.32	0.0	0.0	0.0	0.47
			X̄	0.96	1.53	0.07	0.08	3.99	6.54	0.08	0.10	5.47	8.89
			S.D	1.09	1.01	0.14	0.15	5.14	4.94	0.10	0.13	6.74	6.15
31–40	233	82	Max	10.14	22.97	5.34	0.53	25.77	28.35	0.78	0.54	39.76	54.55
			Min	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.05	0.0
			X̄	1.18	2.01	0.11	0.11	4.22	6.75	0.11	0.09	5.99	9.63
			S.D	1.29	2.79	0.40	0.15	3.54	6.13	0.13	0.12	5.06	9.16
41–50	494	140	Max	18.33	16.93	2.00	4.96	33.50	59.79	2.61	0.64	47.22	70.18
			Min	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			X̄	1.21	2.26	0.07	0.18	4.30	8.61	0.12	0.11	6.08	12.04
			S.D	1.63	2.47	0.18	0.47	3.93	8.58	0.20	0.13	5.71	11.35
51–60	848	154	Max	60.55	90.00	4.74	1.55	39.80	44.58	3.11	3.71	62.76	90.46
			Min	0.0	0.0	0.0	0.0	0.0	0.10	0.0	0.0	0.0	0.16
			X̄	1.22	3.40	0.08	0.16	4.46	9.00	0.13	0.17	6.27	13.58
			S.D	2.29	7.92	0.26	0.26	4.02	8.66	0.20	0.35	5.57	14.14
61–70	1,013	119	Max	90.00	15.00	20.00	2.26	47.20	67.31	3.31	1.25	90.59	89.98
			Min	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			X̄	1.46	2.57	0.12	0.17	4.70	8.11	0.12	0.13	6.82	11.77
			S.D	4.24	2.30	0.91	0.29	4.43	8.02	0.16	0.19	7.21	10.63

TABLE IV.—ADIPOSE RESIDUES UNITED STATES, HUMAN MONITORING DATA, fiscal year 1968; Pesticide Residue Levels in the General Population; Adipose Tissue (p.p.m.); Human Monitoring Survey, Division of Community Studies, OPS, FDA. (*See Figure 3 following these tables.*)—Continued

Age	Sample			ppDDT		opDDT		ppDDE		Dieldrin		Total DDT[1]	
	W	NW		W	NW	W	NW	W	NW	W	NW	W	NW
71–80	813	119	Max	44. 80	90. 00	4. 00	2. 58	51. 34	87. 60	4. 11	4. 62	104. 05	102. 55
			Min	0. 0	0. 0	0. 0	0. 0	0. 0	0. 13	0. 0	0. 0	0. 0	0. 14
			$\bar{X}$	1. 34	4. 07	0. 08	0. 21	4. 52	10. 94	0. 12	0. 17	6. 46	16. 46
			S.D	2. 07	8. 87	0. 21	0. 38	4. 50	11. 94	0. 21	0. 48	6. 72	17. 55
81–90	331	40	Max	20. 00	20. 00	1. 78	2. 29	26. 54	30. 96	0. 89	1. 30	43. 86	40. 01
			Min	0. 0	0. 0	0. 0	0. 0	0. 0	0. 73	0. 0	0. 0	0. 14	1. 03
			$\bar{X}$	1. 34	3. 35	0. 10	0. 22	4. 28	10. 16	0. 10	0. 15	6. 20	14. 89
			S.D	2. 15	3. 59	0. 21	0. 45	4. 04	7. 97	0. 14	0. 24	5. 80	11. 34
90 and over	85	31	Max	17. 50	20. 51	1. 40	1. 85	31. 11	84. 47	2. 38	0. 89	53. 01	109. 05
			Min	0. 0	0. 0	0. 0	0. 0	0. 0	0. 0	0. 0	0. 0	0. 0	1. 92
			$\bar{X}$	1. 56	5. 19	0. 09	0. 32	4. 66	15. 50	0. 14	0. 14	6. 85	22. 78
			S.D	2. 47	5. 61	0. 22	0. 46	4. 87	19. 48	0. 30	0. 23	7. 83	26. 67
Total	4, 165	804											

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[1] Total DDT=ppDDT+opDDT+ppDDE (1.114).

TABLE V.—*Effects of age on total DDT residues in adipose tissue*

Couuntry	Age (years)	No.	Race	DDE	$\overline{X}$ (p.p.m.) total DDT	Dieldrin
Israel (Wasserman, M. et al., 1967).	0–9	71	No difference.	5. 6	10. 2	
	10–89	133	do	9. 9	18. 1	
Florida (U.S.A.) (Davies, J. E. et al., 1968.	0–5	17	White	2. 8	5. 5	
	6+	90	do	5. 5	8. 4	
	0–5	17	Negro	4. 1	7. 8	
	6+	35	do	10. 8	16. 7	
Florida (U.S.A.) (Edmundson, W. F. et al., 1968).	0–5	14	White			0. 23
	6+	88	do			. 23
	0–5	15	Negro			. 21
	6+	29				. 20

Table VI.—*DDT and DDE, in ppb, in whole blood of children and by family unit, in a general population group, Dade County, 1968*

Age	Race and sex	DDT	DDE	Family X DDT	Range DDT	X DDE	Range DDE
1	nw/f	8. 75	4. 33	17. 34	8. 75–23. 75	20. 71	4. 33–39. 46
2	nw/m	19. 48	16. 63				
4	nw/f	16. 27	23. 52				
5	nw/f	18. 28	20. 61				
6	nw/m	23. 75	39. 46				
Area:	South Miami.						
2	nw/f	10. 96	8. 35	12. 83	9. 17–21. 36	9. 93	7. 23–11. 67
3	nw/m	21. 36	11. 37				
4	nw/m	9. 83	7. 23				
5	nw/m	9. 17	11. 67				
Area:	Richmond Heights.						
1	nw/m	52. 97	45. 81	36. 17	21. 58–52. 97	31. 63	14. 62–45. 81
2	nw/f	48. 55	43. 32				
3	nw/f	21. 58	14. 62				
5	nw/f	21. 58	22. 78				
Area:	Homestead.						
2	nw/m	70. 04	69. 42	63. 9	51. 11–70. 55	54. 76	37. 71–69. 42
3	nw/m	70. 55	57. 15				
5	nw/m	51. 11	37. 75				
Area:	Goulds.						
2	nw/f	5. 83	4. 73	6. 45	4. 58– 9. 07	7. 5	4. 73–11. 67
4	nw/m	4. 58	6. 22				
5	nw/f	9. 07	11. 67				
Area:	Richmond Heights.						
4	w/m	7. 34	9. 55	3. 67	<4. 02– 7. 34	7. 81	6. 07– 9. 55
5	w/m	<4. 02	6. 07				
Area:	Homestead.						
2	nw/m	<5. 85	3. 97	7. 49	<5. 85– 9. 13	6. 75	3. 97– 9. 53
4	nw/m	9. 13	9. 53				
Area:	Richmond Heights.						
5	w/f	4. 02	11. 74	4. 74	4. 02– 5. 47	12. 79	11. 74–13. 85
6	w/f	5. 47	13. 85				
Area:	Miami.						
3	w/m	<2. 26	2. 48	2. 57	2. 18– 2. 68	4. 36	2. 48– 6. 58
4	w/f	2. 68	4. 03				
6	w/f	2. 18	6. 58				
Area:	Goulds.						

FIGURE 3.—*Adipose levels of DDE (ppm) by age and race, U.S. 1968.*
(Data Human Monitoring Program)

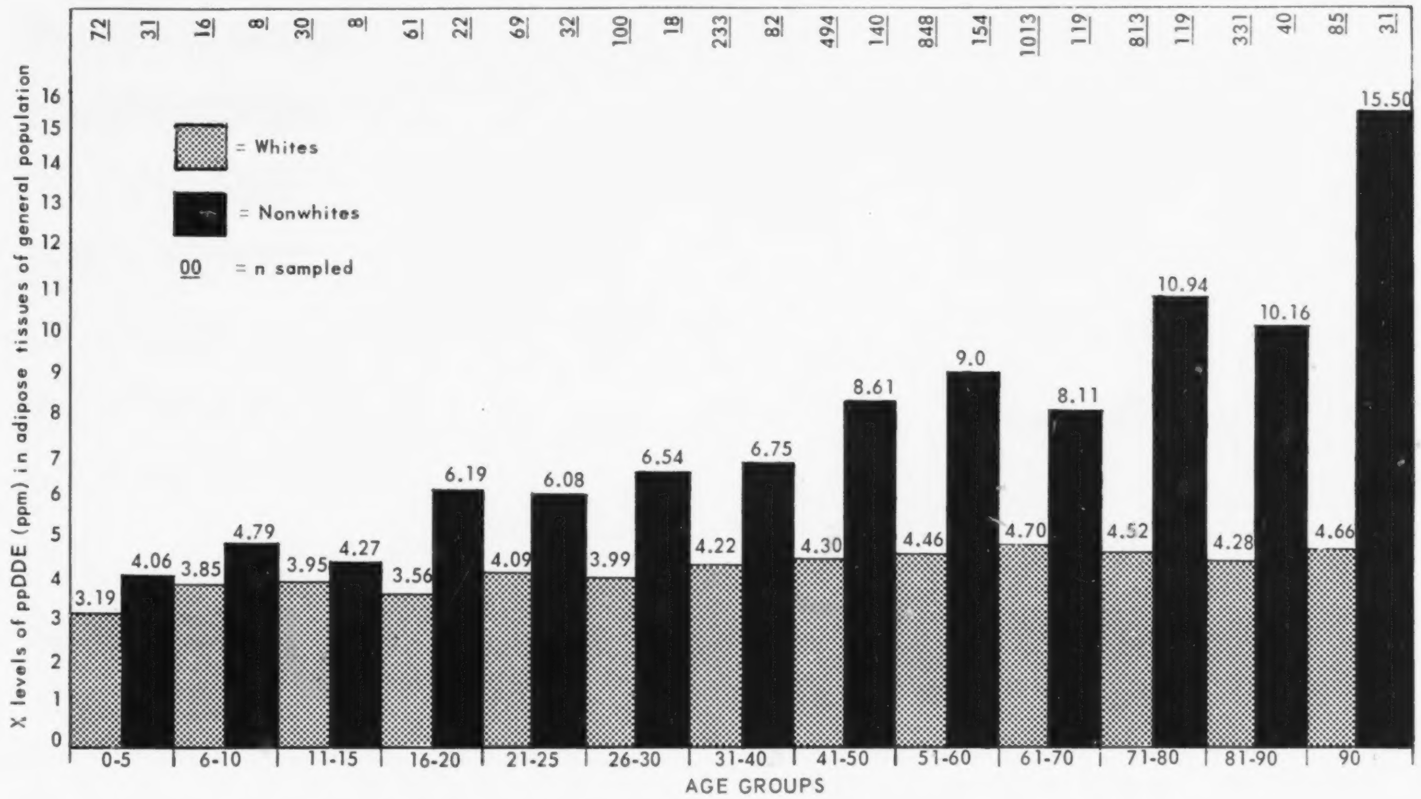


FIGURE 4.—*Comparison of human adipose residues (ppm) of total DDT and dieldrin, by age and race in northern and southern states, U.S.A., 1968.*

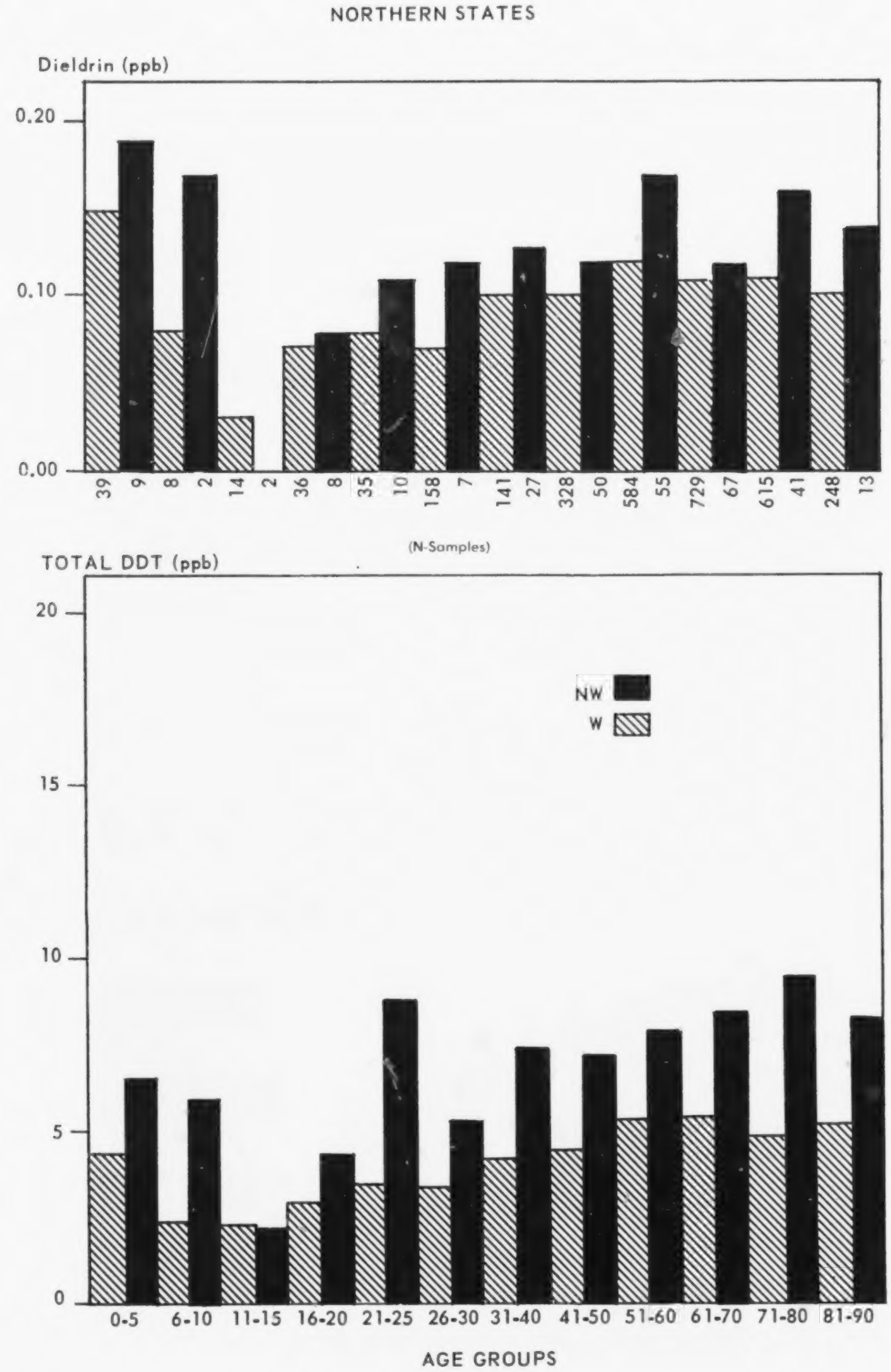
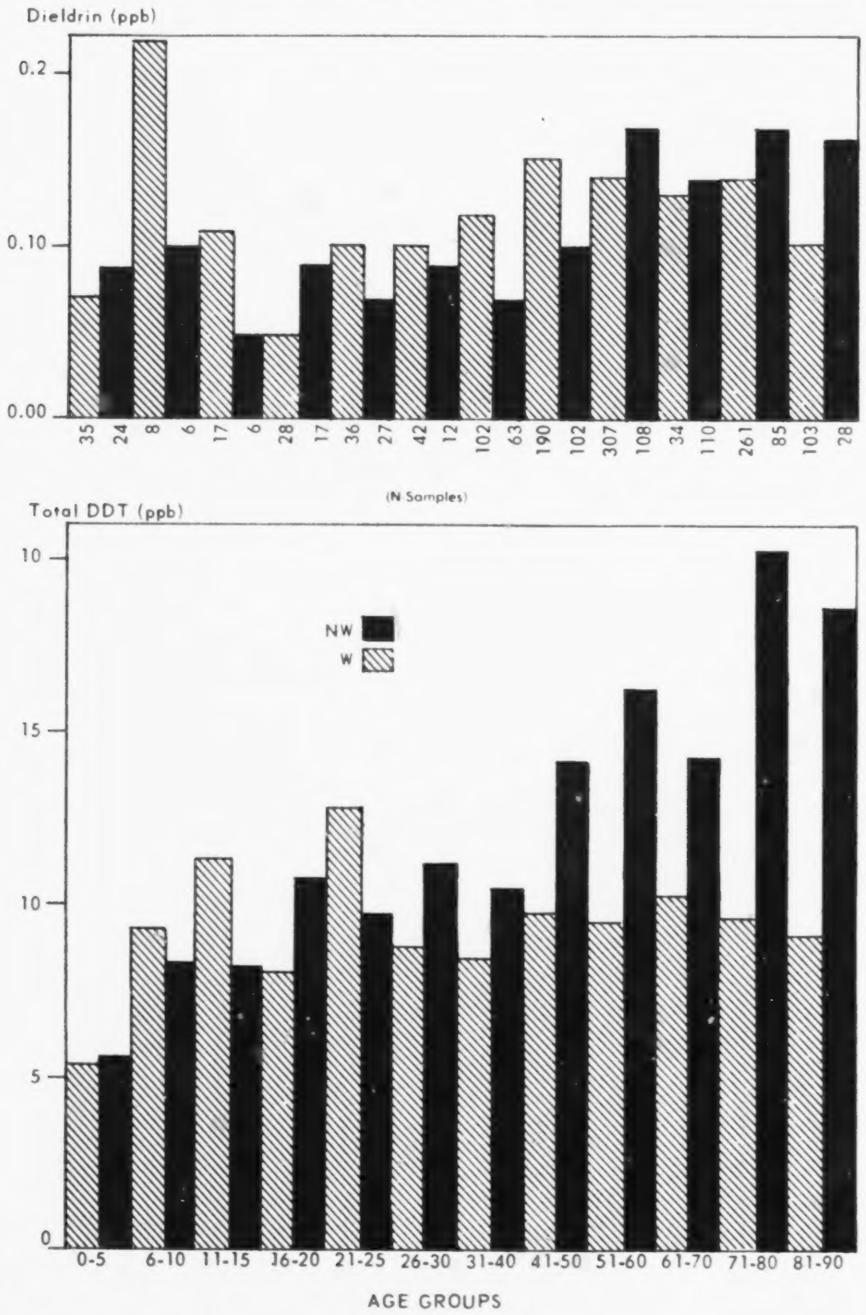
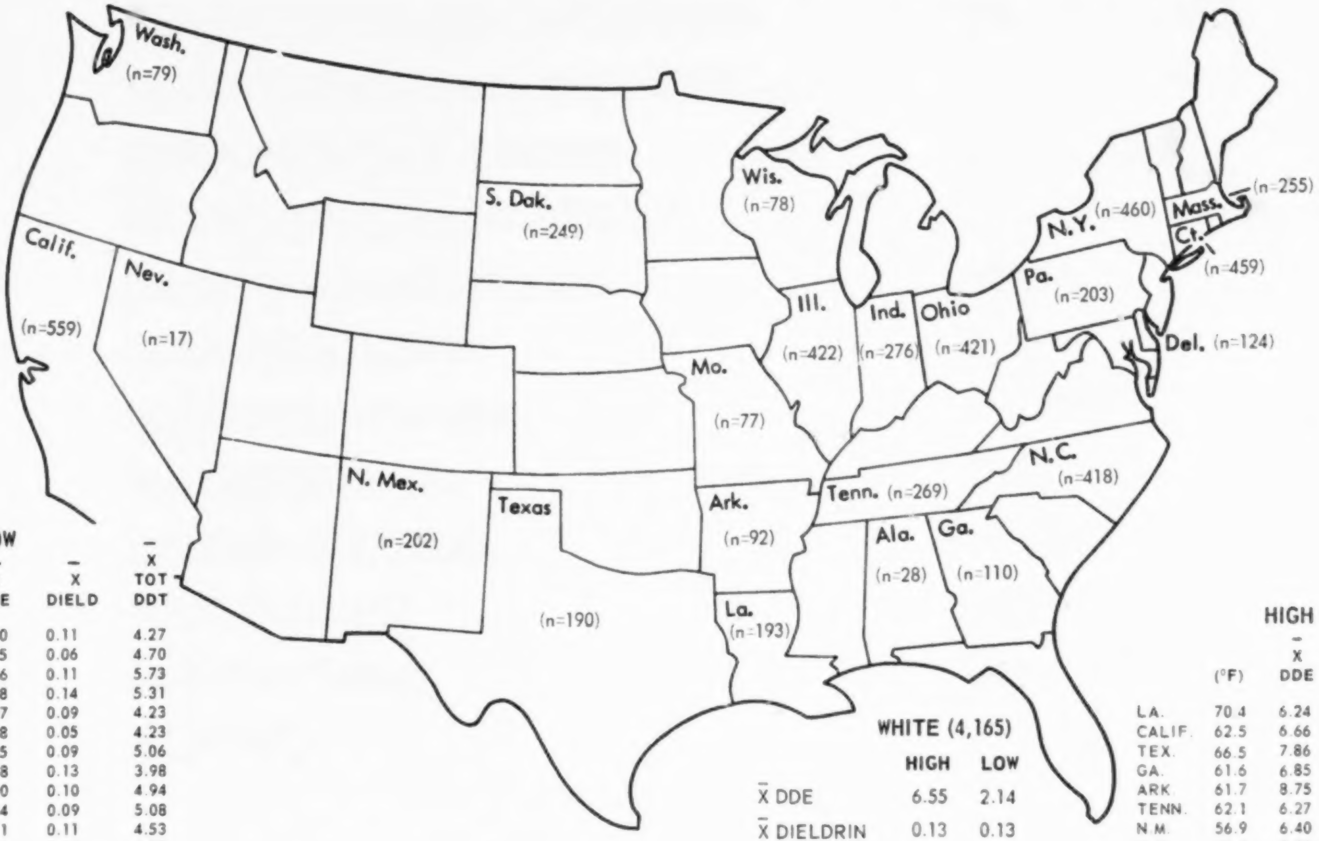


Figure 4.—*Comparison of human adipose residues (ppm) of total DDT and dieldrin, by age and race in northern and southern states, U.S.A., 1968. (cont'd)*



SOUTHERN STATES

Figure 5.—*Adipose residues of p,p' DDE (ppm), dieldrin and total DDT in general population U.S.A. human monitoring data fiscal year 1968.*


Wash. (n=79)
Calif. (n=559)
Nev. (n=17)
S. Dak. (n=249)
N. Mex. (n=202)
Texas (n=190)
Mo. (n=77)
Ark. (n=92)
La. (n=193)
Wis. (n=78)
Ill. (n=422)
Ind. (n=276)
Ohio (n=421)
Tenn. (n=269)
Ala. (n=28)
Ga. (n=110)
N.C. (n=418)
Pa. (n=203)
N.Y. (n=460)
Mass. (n=255)
Ct. (n=459)
Del. (n=124)

LOW

	(°F)	$\bar{X}$ DDE	$\bar{X}$ DIELD	$\bar{X}$ TOT DDT
MO.	56.0	2.90	0.11	4.27
WASH.	53.2	2.95	0.06	4.70
S.D.	45.7	3.66	0.11	5.73
CONN.	49.3	3.48	0.14	5.31
MASS.	51.4	2.97	0.09	4.23
NEV.	48.8	2.98	0.05	4.23
IND.	52.1	3.45	0.09	5.06
ILL.	50.9	2.78	0.13	3.98
N.Y.	54.5	3.50	0.10	4.94
OHIO	55.2	3.44	0.09	5.08
PA.	54.6	3.11	0.11	4.53
DEL.	54.1	2.96	0.09	4.38
WISC.	45.1	3.97	0.15	5.53

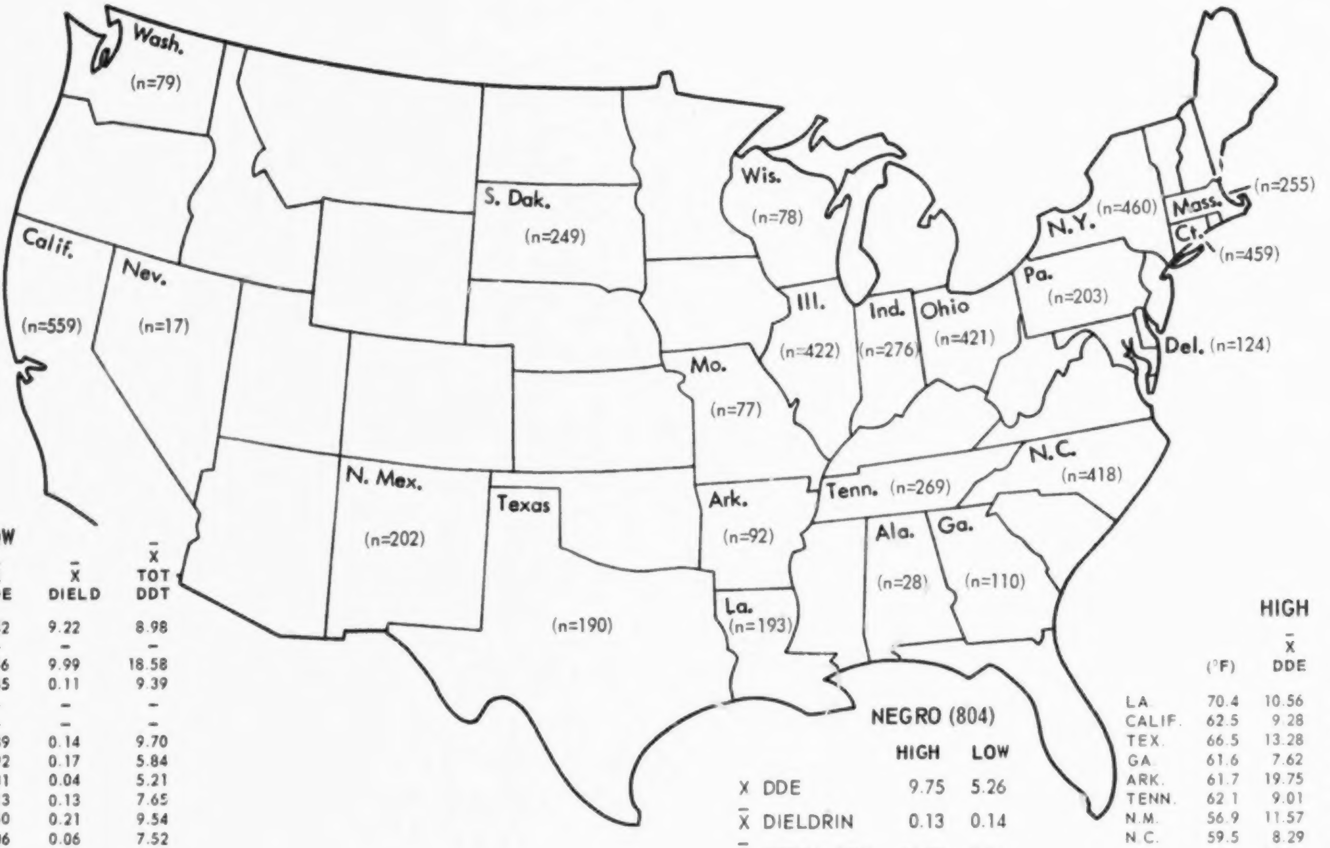
WHITE (4,165)

	HIGH	LOW
$\bar{X}$ DDE	6.55	2.14
$\bar{X}$ DIELDRIN	0.13	0.13
$\bar{X}$ TOTAL DDT	9.21	4.85

HIGH

	(°F)	$\bar{X}$ DDE	$\bar{X}$ DIELD	$\bar{X}$ TOT DDT
LA.	70.4	6.24	0.10	8.55
CALIF.	62.5	6.66	0.16	9.35
TEX.	66.5	7.86	0.21	10.73
GA.	61.6	6.85	0.18	9.68
ARK.	61.7	8.75	0.21	13.23
TENN.	62.1	6.27	0.17	9.28
N.M.	56.9	6.40	0.14	8.50
N.C.	59.5	5.50	0.11	7.73
ALA.	67.2	4.90	0.01	5.92

FIGURE 6.—*Adipose residues of p,p' DDE (ppm), dieldrin and total DDT in general population U.S.A. human monitoring data fiscal year 1968.*


Wash.
(n=79)
Calif.
(n=559)
Nev.
(n=17)
S. Dak.
(n=249)
N. Mex.
(n=202)
Texas
(n=190)
Wis.
(n=78)
Mo.
(n=77)
Ark.
(n=92)
La.
(n=193)
Ill.
(n=422)
Ind.
(n=276)
Ohio
(n=421)
Tenn. (n=269)
Ala.
(n=28)
Ga.
(n=110)
N.C.
(n=418)
Pa.
(n=203)
N.Y. (n=460)
Mass.
(n=255)
Ct.
(n=459)
Del. (n=124)

LOW

	(°F)	$\bar{X}$ DDE	$\bar{X}$ DIELD	$\bar{X}$ TOT DDT
MO.	56.0	5.82	9.22	8.98
WASH.	53.2	–	–	–
S.D.	45.7	14.56	9.99	18.58
CONN.	49.3	6.85	0.11	9.39
MASS.	51.4	–	–	–
NEV.	48.8	–	–	–
IND.	52.1	6.39	0.14	9.70
ILL.	50.9	3.92	0.17	5.84
N.Y.	54.5	3.31	0.04	5.21
OHIO	55.2	5.13	0.13	7.65
PA.	54.6	6.50	0.21	9.54
DEL.	54.1	5.06	0.06	7.52
WISC.	45.1	4.26	0.20	6.11

NEGRO (804)

	HIGH	LOW
X DDE	9.75	5.26
$\bar{X}$ DIELDRIN	0.13	0.14
$\bar{X}$ TOTAL DDT	14.37	7.86

HIGH

	(°F)	$\bar{X}$ DDE	$\bar{X}$ DIELD	$\bar{X}$ TOT DDT
LA.	70.4	10.56	0.06	14.75
CALIF.	62.5	9.28	0.16	12.76
TEX.	66.5	13.28	0.27	18.64
GA.	61.6	7.62	0.19	11.70
ARK.	61.7	19.75	0.10	28.86
TENN.	62.1	9.01	0.08	14.14
N.M.	56.9	11.57	0.38	15.15
N.C.	59.5	8.29	0.11	13.21
ALA.	67.2	10.20	0.00	11.36

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Clinical Case Reports

Mortality and morbidity studies.—Other studies have sought to explore the long term effects of pesticides on man by comparison of mortality and morbidity in population groups whose differential pesticide exposure is measured, not by residue level, but by reason of exposure variations associated with person (occupational studies), place (urban, rural differences, proximity to agriculture), and time (incidence rates per and post DDT eras, summer, winter variations).

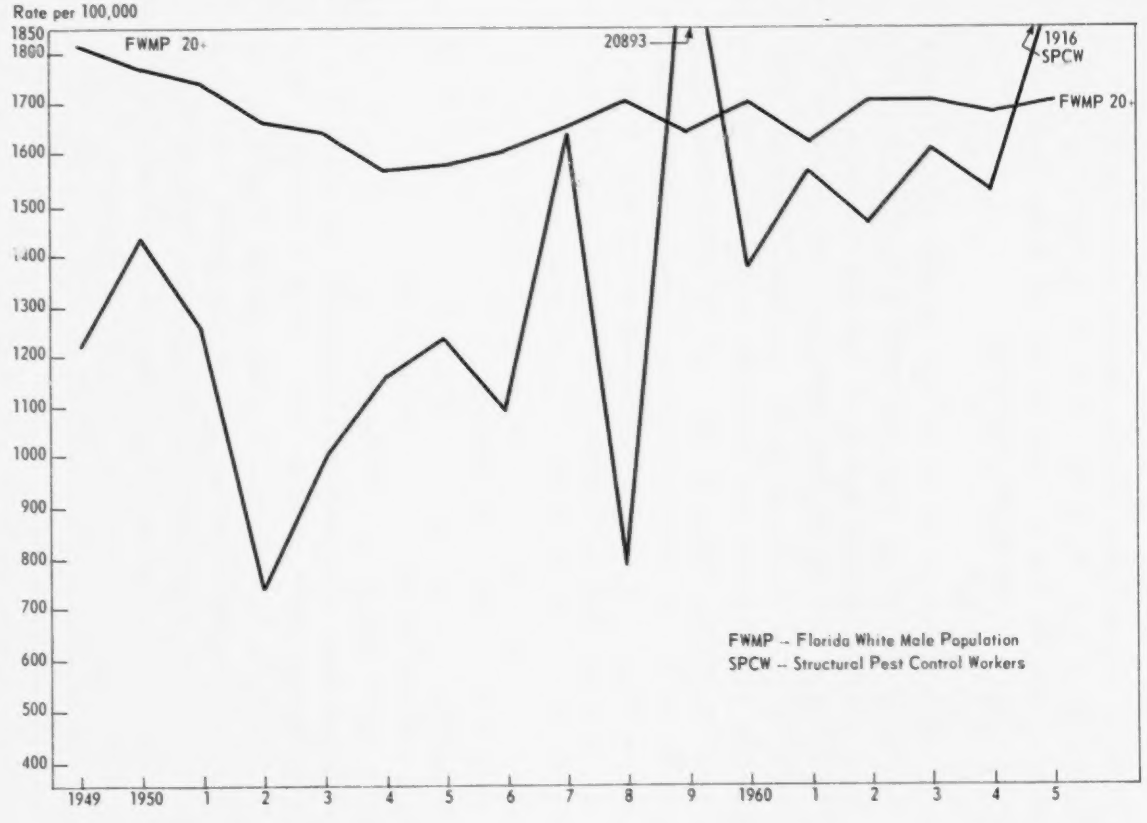
Mortality studies.—One of the only comprehensive reviews of mortality data on persons occupationally exposed to pesticides is the retrospective mortality study of Florida registered structural pest control workers, who by law had to register with the Florida State Board of Health beginning 1948 (Boorde & Downes, 1969). This presents mortality data for the years 1948–65 made up of more than 20,000 person-years of observation comparing their mortality rate trends with the rate of the Florida white male population 20 years old and over. The age-adjusted mortality rates of this single occupational group showed a rising death rate for structural pest control workers against a fairly stable age-adjusted rate for the entire population (Figure 7). It should be pointed out that even during the last few years of this study, the age-adjusted rate for the Florida white male population were still somewhat above rates for pest control workers. This could be interpreted as an improvement in the quality of study data rather than a rising death rate among the study group.

(Figure 7 is on p. 346.)

On the other hand, a review of age specific rates for pest control workers 35–44, the group with the most years of observation, shows they have a death rate in excess of that for the same Florida white male age group. Among another pivotal age group, 55–64, age specific death rates for the study group are practically on the same level as the Florida white males and it would be expected that the general population mortality rates would significantly exceed rates for any supposedly healthy occupational group. These data were derived from a mailed questionnaire survey which had a nonresponse rate of 32 percent. An intensive followup effort in one county suggests the mortality experience of the nonrespondents was approximately the same as the respondents. If this assumption is made the survey suggests that long term exposure to pesticides may have an adverse chronic effect on human health and supports the need for continued view of possible health hazards. Comparisons of causes of death for 180 structural pest control workers against a matched control group showed no significant differences in cause distribution. If pesticides are a health hazard and no single disease entity can be demonstrated as being casually associated we may be dealing with a phenomenon through which an increased risk of mortality exists for a whole spectrum of diseases. Such a situation would greatly complicate epidemiological research on this subject. From any point of view additional prospective morbidity and mortality studies are required to prove or disprove cause and effect relationship between specific pesticides and disease classifications.

Morbidity effects of occupational exposure.—Numerous morbidity surveys have been conducted in workers exposed to a single or group of pesticides. Thus, for the organophosphate compounds, the health effects of occupational exposure for the following pesticides have been described: azinphos-methyl (Simpson, 1965), demeton (Kagan et al., 1958), dichlorvos (Witter, 1960; Gratz et al., 1963; Funckes et al., 1963; Stein et al., 1966; Zavon & Kindel, 1966), fenitrothion (Vandekar, 1965), fenthion (Taylor, 1963), malathion (Grech, 1965) methyl

FIGURE 7.—*Comparison of Florida white male population, 20 years and older with structural pest control workers registered in Florida 1948–1965 by age-adjusted* mortality rates per 100,000. Florida State Board of Health.*



*Indirect Method. Standard: FWMP 204. July 1, 1960 demeton (Asribekova, 1963), parathion (Brown & Bush, 1950, Ingram, 1951; Kay et al., 1952; Lieben et al., 1953; Simpson & Beck, 1965; Davies et al., 1965), thiometon (Rosival & Rajnoha, 1961). For the carbamate compounds: carbaryl (Best & Murray, 1962; Vandekar, 1965), 3-isopropylphenyl N-methylcarbamate (Vandekar, 1965). For the chlorinated hydrocarbons: DDT (Krasnyuk, 1958; Ortelee, 1958; Krasnyuk, 1964; Quinby et al., 1965; Laws et al., 1967; Edmundson et al., 1969^{a}; Edmundson et al., 1969^{b}; Edmundson & Davies, to be published); BHC (Bogushevskii & Burkatskaya, 1951; Brzozwski et al., 1954; Burkatskaya et al., 1959), chlorodane (Alvarez & Hyman, 1953; Fishbein et al., 1964), dieldrin and related compounds (Fletcher et al., 1959; Hoogendam et al., 1962, 1965; Van Raalte et al., 1970), p-dichlorobenzene (Pagnotto & Walkley, 1965), dichloroethane (Brzozowski et al., 1954), trichloroethane (Frant & Westendorp, 1950). For combination of compounds: Barnes & Davies, 1951, Princi & Spurbeck, 1951; Fowler, 1953; Sumerford et al., 1953; Culver et al., 1956; Bruaux, 1957; Hayes et al., 1957; Paulus et al., 1957; Quinby et al., 1958; Vengerskaya et al., 1959; Wasserman et al., 1960; Klhufkova & Pospisil, 1961; Lyubetskii & Vengerskaya, 1961; Ruprich, 1961; Stein & Hayes, 1964; Davignon et al., 1965; Hartwell & Hayes, 1956). The results of these health surveys can be discussed under the categories of organophosphates, carbamates, organochlorine insecticides and herbicides.

Organophosphates—The organophosphates particularly parathion, phosdrin, and TEPP are health hazards because of their high toxicity and ease of absorption by ingestion and the dermal or respiratory routes. The disease they produce in workers are chiefly acute poisonings, but may in addition leave sequelae of acute intoxication which are probably anoxic sequelae. However, there is an additional hazard to workers which may not be recognized; this is because undue exposure resulting in cholinesterase inhibition from chronic exposure may predispose the worker to poisoning when he is exposed subsequently (to an exposure not expected to be noxious) with the result of clinical signs of poisoning. Somewhat similar hazards occur when pesticides in plants e.g. orchards build-up slowly from repeated spraying with residues of organiphosphates to the point that orchard workers are affected gradually by dermal or respiratory contact with the plant material (Milby, 1964).

Continued long term exposure to organophosphates appear to have other subtle effects. Evidence has been presented to alter renal tubular functions in a small percentage of workers when measured by phosphorous reabsorption tests (Mann, J. B. et al., 1966). These findings were attributed to renal tubular damage from the metabolite paranitrophenol. A change in protien metabolism as indicated by a modest increase in amino acid levels as measured in blood serum may be reflective of liver damage or more probably represent an adaptive response on behalf of the liver, in response to enzyme induction changes necessary for the general detoxification of pesticides (Davies, J. et al., 1969; Tocci, P. M. et al., 1969). In other studies, chronic exposure to organophosphates were considered to produce slow reaction times, memory defects and to cause workers to become increasingly irritable; motor or sensory defects were not observed but electroencephalographic studies revealed more than usual incidence of abnormalities (Metcalf, 1969).

Carbamate Exposure—Cases of obvious carbamate poisoning are uncommon, and are not usually associated with occupational exposure. Undoubtedly many intoxications occur but these are usually mild and are unreported since they involve workers in the initial stages of exposure to these chemicals (inception illness); as adaptation occurs, symptoms usually subside. Carbaryl (Sevin) is the most commonly used of the carbamate insecticides and causes few problems in the course of application. Baygon$^{(R)}$, a longer acting carbamate can produce mild toxicity symptoms especially in persons spraying the solution under conditions which predispose to excessive exposure (Vandekar, 1965; Davies, 1968 (personal communication)). In mosquito control programs small domestic animals and children may become affected by the concentration of the chemical in the house and become ill in a samilar manner, but again symptoms are mild and temporary and recovery is the rule. The carbamates have a reversible inhibition of cholinesterase so that laboratory determinations must be done rapidly and interpreted with caution. The clinical picture of poisoning by carbamates is similar to that produced by the organophosphates but in contrast may be made worse with 2–PAM (Hayes, 1963).

DDT Studies—In recent study of 35 men with experience from 11–19 years in a plant producing DDT exclusively (average 6 million lbs./month). no ill effects attributed to DDT were observed among the workers (Laws et al., 1967). The fat storage levels of total DDT-derived materials ranged from 38–647 p.p.m. as compared to 8 p.p.m. for the general population. Serum values of total DDT were also high and correlated with fat values ((r)=0.64). A review of plant records of 63 men with more than 5 years exposure likewise was not fruitful in revealing diseases or unusual frequency of poisoning due to DDT.

No evidence of adverse effects was seen in a study of 40 workers in DDT formulating plants (average work duration was 3.5 years and exposure was to other chemicals as well) (Ortelee, 1958), nor was disease identified in a study of volunteers who were fed up to 35 mgms. of DDT per day for 21 months (about 200 times the normal dietary intake of 0.0026 mg./kg./day). The storage of DDT (but not DDE) was proportional to dosage and attained a constant level, despite dosage, after 1 year or more (Hayes et al., 1956).

Elsewhere in a study of 400 persons exposed to organochlorine pesticides it was suggested that these workers experienced a higher frequency of cardiovascular diseases of various types than was expected; the conclusion was reached based on electrocardiographic changes in persons working with organochlorine insecticides (Krasynuk, 1964).

The Diene Group—aldrin, dieldrin, endrin.

Studies have been reported in plants manufacturing these insecticides. Seventeen patients (5 percent of the work force) developed epileptiform convulsions (Hoogendam et al., 1965). Efforts were made to pinpoint the hazard; these were noninformative. Potential dermal exposure by estimation of dose with dermal absorbent pads were not considered worthwhile. Abnormalities of the encephalogram were demonstrated in the cases; these were always temporary and were associated with specific incidents of overexposure. No cases of permanent, partial or complete incapacity were recorded and no claims for compensation were filed. Rates of sickness, observation, skin sensitization reactions, abnormalities of liver function tests were no different in 300 workers studied than in the nonpesticide workers, and the occurrence of epileptiform convulsions in the 17 cases was the only abnormality demonstrated. A biologic index of intoxication (concentrations of dieldrin in the blood of 15 μg/100 g.) appeared to be the threshold level denoting the appearance of symptoms of intoxication and the procedure was recommended both as a diagnostic test and a surveillance tool (Brown et al., 1964).

One of the same investigations carried out on human volunteer experiment using doses representative of normal adventitious exposure (estimated 0.025 mg.m./day and occupational exposure 0.21 mg.m./day) for 18 months. No signs of ill health were demonstrated and laboratory investigation which included alkaline phosphatase, RBC and plasma cholinesterase, EEG, ECG and electromyographic studies were all within normal limits. Dieldrin concentrations in blood and fat were proportional to daily dosage and were up to 10 times greater than general population levels. There was no significant effects on the total DDT in fat nor were there changes in the DDE levels in blood. It was concluded that dieldrin in doses of 200 μg./day were without effects on humans (Hunter and Robinson, 1967).

In another study of 71 men of which 49 were production workers (in a plant manufacturing dieldrin, aldrin, endrin and organophosphates) no meaningful correlation of sick leave with dieldrin levels in fat, blood and urine was demonstrated. Concentrations of dieldrin in fat reached levels more than 19 times the level of the general population and blood levels were more than nine times greater than general population blood levels. A correlation of levels in blood with total duration of exposure was demonstrated and blood samples were recommended for monitoring workers (Hayes and Curley, 1968).

Other Chlorinated Hydrocarbons—Other chemicals of this class have been suspected of causing adverse health reactions. Lindane has been incriminated directly or indirectly with 18 cases of blood dyscrasias, and the California Board of Health, and more recently the U.S. Department of Agriculture have passed resolutions recommending against the use of lindane vaporizers. There is an admitted lack of technical data on which to base sound regulations but the suspicion that lindane is a hazardous chemical (West, 1967). A recent unreported study of 70 persons highly exposed to lindane demonstrated slight changes in blood when compared to matched controls (elevated polymorphonuclear, and total white count, and slightly reduced creatinine) (Milby, 1969).

In a factory manufacturing phenoxy chemicals (the herbicides—2–4D, 2, 4, 5 T) a number of workers were found to have hyperpigmentation and fragility of the skin, eruptions on exposed areas and acne lesions. Porphyria was observed in 37 percent of the 29 workers tested. It was postulated that the disturbed metabolism was due to a toxic action on the liver by one or more different chemicals being used in the factory although no direct relationship between clinical signs as the degree of chemical exposure was demonstrated; the diseases were considered to be the result of individual susceptibility (Bleiberg et al., 1964). Porphyria has also been described following ingestion of hexachlorobenzene (Schmid, 1960). In a group of 250 agricultural workers and 45 workers engaged in crop spraying for 3 years using 2,4–D, excessive fatigue was reported. In addition, they complained of headaches, epigastric pains, anorexia and occasional upper respiratory tract symptoms and several demonstrated impaired taste sensitivity (Fetisov, 1966).

Apart from occurrence of overt disease as expression of effect, the Community Pesticide Studies Program of the U.S.P.H.S. is currently involved in a 16 State collaboration research, wherein prospective morbidity data is being collected from groups of occupationally exposed workers. The incidence of disease as well as biocemical differences is being studied. Although as yet, no correlation of data is available, an impression is being gained of the occurrence of certain biochemical changes in occupationally exposed groups when compared with controls.

Thus, from Hawaii, in studies of 59 persons occupationally exposed to a wide variety of pesticides, significant differences in hematocrit, white blood count, serum albumin, alkaline phosphatase, potassium and PBI (protein bound iodine) were observed. Gastrointestinal disturbances, obesity, diabetes, thyroid and neurologic dysfunctions were the principal findings.

In Iowa (Long et al.), demonstrated significant correlation of hematocrit, hemoglobin and prothrombin values with high pesticide usage. Significant correlations were found between total pesticides employed and the blood uric acid and bilirubin 1-minute values (Long et al., 1969).

In Florida, levels of certain biochemistries, reflective of kidney or liver function changes were observed in 67 persons heavily exposed to pesticides when compared with healthy normal controls. The exposed group showed unusually high levels of several amino acids, SGOT, alkaline phosphatase, serum osmolality and creatinine (Tocci et al., 1969).

Thus by interstate collaborative research, changes of certain biochemical parameters are beginning to observe in large groups of persons occupationally exposed to pesticides. Several notes of caution should be respected with these types of findings. Firstly, differences are mostly within the normal range of the biochemical parameters, and thereby are not considered pathological, secondly, associations only has been demonstrated and no causal inference concluded, thirdly, criteria of pesticide exposure varies from State to State and uniform methods of degrees of pesticide exposure between States is difficult if not impossible and lastly, some of the biochemical changes observed may be adaptive or compensatory rather than reflective of an adverse health effect so that changes rather than disease entites are being reported.

In concluding the section of review of studies of the occupational exposed group, an illustration of the epidemiologic worth of such studies can be seen from the findings of a medical survey of persons working with aldrin and dieldrin (Jager, 1969).

In this excellent study of 826 plant employees, detailed morbidity data are presented on 233 persons representing 1,728 years of exposure to aldrin and dieldrin including significant data on exposure; diseases included glomerulonephritis (1) peptic ulcers (3) nephrolithiasis (4) ischaemic heart disease (2) reticulosarcoma (1) carcinoma of the stomach (1) and glioma (1). In addition, an intensive medical survey of highly exposed workers revealed no significant incidence of any disease occurring in the plant when compared with findings from employees less exposed in the plant. Thus, the data supported the concept that hazards of working with pesticides were slight. However, interpretation of the incidence rates of the above mentioned diseases which were seen to occur, is difficult and calls for comparison with incidence rates of the general population. If our interest is the investigation of diseases with long incubation periods, such as cancer, then this approach will have to be followed in future studies. The problem of tracing dropouts is also exemplified and demonstrates the ease of using occupational studies as measures of occupational risk, but the complexity of such studies when used to extrapolate as to health effects of the pesticide exposure of the general population.

Miscellaneous general population studies.—The literature contains examples of several studies which have explored place and time variables as possible indications of greater pesticide exposures. Thus, increases in upper respiratory diseases, including asthma, sinusitis and bronchitis have been associated with the greater domestic usage of pesticides in Hawaii (Weiner and Worth, 1969). Because of the lindane findings due to neoplasia by lymphatic and hematopoietic tissues and aplastic anemia in Kern County were compared with the total California experience (Mengle et al., 1967). No significant differences were observed in these two areas. Ganelin has explored the effects of incidental exposure to parathion on the general population and asthmatics (Ganelin et al., 1964). Their data suggested that the effects of such incidental exposure was negligible.

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Conclusion and recommendations.—An epidemiologic appraisal of the total health effects of pesticides occurring under the categories of acute, general population and occupationally exposed recognizes the adverse health effects or areas of uncertainties in all three exposure situations. Recommendations are suggested with the intent of preventing these adverse effects or in the hope of obtaining greater information in those areas where gaps in our knowledge seem to exist.

The summation of the findings resulting from acute pesticide exposure indicate that in the United States, the main offenders are the more toxic of the organophosphates. More poisonings can be expected with greater usage of this group. Children and adults are the two different population groups involved. The former reflect the readily reachable chemicals in the home. Their prevention is dependent upon such factors as the better public awareness of their hazards, improved legislation prohibiting the more toxic members from home use, industrial improvements in packaging and labeling, and in the case of roach baits, substitution of the more toxic baits by less toxic but equally effective substitutes.

Disregarding the suicide component of pesticide intoxications, adults are most frequently poisoned accidently in the home or while at work. At home the problem is often the result of storage in improper containers and the answer again is a better awareness. Occupationally, the pesticide industry demonstrates a steady improvement with regard to acute poisoning. The virtue of protective clothing and other protective devices have been well disseminated—the dangers often resulting from the employment of transient laborers, such as high school students on their summer vacation, or workers who cannot read or do not understand the nature of the toxicity of the chemicals with which they are working. There is great need for field investigation of the more serious cases and facilities are suggested for better investigation, documentation, and reporting of such events. An areawide pesticide protection team is suggested to this end, whose members would be drawn from representatives of the local health departments, the country agricultural agent and a representative of Fish and Wildlife. Episodes of human and wildlife poisoning would be investigated. In addition, advice and recommendation toward the design and disposal of containers, occupational aspects of safe handling of pesticides would be promoted.

In the area of occupational exposure two conclusions can be reached. The first relates to surveillance. With improved technology the occupational hazard to the worker is decreasing. Cholinesterease testing is known to prevent overt and detect incipient toxicity. In manufacturing situations, formulation and antimalarial spraying, clinical surveillance against organochlorine toxicity or excessive exposure is now practicable and probably worthwhile; particularly is this so with dieldrin and could be extended to DDT surveillance. In view of the possible consequences of enzyme induction in situations of extreme exposure to DDT, a ceiling for DDT blood levels might be determined if significant enzyme induction is demonstrable and proved harmful. The second conclusion relates to future research in this area and is based upon the findings of the increasing age-adjusted mortality rates observed in structural pest control operators. These findings together with the information of abnormal chemistries from several of the Community Studies Programs warrant continued intensive clinical and biochemical surveillance of selected groups of high-exposed pesticide operators. The main function of such investigations would serve to determine whether a chronic occupational hazard exists from exposure to cetrain selected pesticides.

Insofar as the general population is concerned three recent events have occurred which have prompted a resurgence of public concern of the health effects of the persistent pesticides on mankind and his environment. The first of these was the occurrence of obvious adverse ecological effects of pesticides on certain predator birds and the high levels in certain fish (Coho salmon). The second stems from the demonstration of enzyme induction by DDT and the uncertainty of the human consequences of the adaptive mechanism. The third is the recent findings of the Bionetics report (Innes, J. R. M. et al, 1969) wherein carcinogenic potential of DDT and several other insecticides has been demonstrated in mice.

This review of the literature on the effects of incidental exposure to persistent pesticides, and to DDT in particular, indicate that the only certain demonstrable effect of this exposure in man is the tissue residue. What the data also suggests is that we do not fully understand the epidemiology of these levels and perhaps that some of the several assumptions that have been made need to be reappraised. Thus, the significantly higher levels of DDT-derived residues in the Negro in the United States necessitates a deevaluation of conclusions which did not adjust for this difference; without straification we cannot describe the average residue level in the country. The age association of levels of DDE in all age groups certainly requires further investigation and explanation. The geographical stratification conflicts with the concept of the dominant role of dietary sources of DDT. If nondietary sources are of significant consequence, then monitoring of human residues will assume as great an importance as food monitoring for these biological residues are the most important expressions of the magnitude of the environmental contamination. Finally, there appears to be so many areas which require further investigation and exploration, and since in the United States we have no need to use as much DDT and as indiscriminately as we have done so in the past, few will question the merit of striving to reduce or at least contain the amount of human and animal contamination that results from this insecticide. Research endeavors in all areas which strive to further the reduction of this environmental contamination seem warranted and timely, and it should not be necesary to have to wait for proof that the exposure is harmful before some action is taken.

Clinical case reports

In the past 25 years, thousands of reports of human illness apparently caused by pesticides have appeared in the medical literature of dozens of countries. An effort has been made here to organize representative examples of this vast body of information in a coherent way. Where possible, findings have been critically assessed, and those which appear scientifically tenable have been arranged according to the human organ system which seemed most affected, or according to a disease entity or syndrome when that was more appropriate.

Case reports in which pesticides are implicated typically describe clinical observations of an individual patient (occasionally more than one), pathological findings, etiology or causal relationships, and a regimen of treatment and its outcome.

Whenever information permits, a consideration of dose-response relationships is included here. However, clinical case reports, by their very nature rarely provide an opportunity for clear documentation of exposure factors. Characteristically, the patient does not seek medical attention until his illness is overtly manifested and he often is unable to relate how much toxin was ingested, inhaled, or absorbed or to accurately reconstruct the events leading to the poisoning.

The clinical picture of acute pesticide intoxication may vary in severity from quite mild to critical or even fatal. Depending upon the agent involved, the diagnosis may be clear-cut or may be exceedingly difficult to firmly establish. The diagnosis is often most difficult in the case of mild to moderate acute poisoning by the organochlorine group of insecticides. This is particularly true when exposure factors are not well documented. The criteria upon which a diagnosis of organochlorine intoxication may be convincingly based are not clearly established. Marked elevations in serum organochlorine levels with or without alterations in electroencephalographic patterns, if present, may provide the only objective clinical evidence of chronic poisoning. Subjective evidence such as irritability, insomnia, anorexia, headache, nausea, malaise may be extremely difficult to evaluate in instances of low level organochlorine exposure.

Because of these diagnostic complexities, unproved or specious *post hoc, ergo propter hoc* associations attributing human illness to pesticide exposure are not uncommonly encountered in both the lay press and the scientific literature. Errors of omission may be equally as serious as errors of commission. The reporting physician, for example, may have failed to study some organ system, such as the central or peripheral nervous system, thereby failing to observe and report a response which did, in fact, occur. Once errors of either commission or omission find their way into print, they are often exceedingly difficult to correct.

Effects on the nervous system.—It is evident from the acute effects of chlorinated insecticides, organophosphate and carbamate insecticides on mammals that these compounds exert their characteristic toxicity by affecting the nervous system. There is evidence of both peripheral and central effects.

The nervous system effects of acute DDT poisoning have been reviewed by Hayes (1959) in an extensive treatise on that compound and von Oettingen (1955) has reviewed these effects for most of the chlorinated insecticides in his book on the halogenated hydrocarbons. Koelle (1963) has edited an extensive volume on the effects of anticholinesterase on the nervous system and both Heath (1961) and O'Brien (1960, 1967) has each published a book on this subject. However, there is no review on the nervous system effects of low-level prolonged exposure to insecticides. This is primarily due to the fact that there are very few publications to be reviewed in this area.

Chlorinated hydrocarbons: There is extensive evidence that the acute action of chlorinated insecticides, particularly DDT, can be detected under certain conditions in the cerebrum, cerebellum, brainstem, spinal cord, and peripheral nerves (Bromiley and Bard, 1949; Shankland, 1964). Thus the acute poisoning effects of these compounds appear to be a diffused nervous system effect thought to be caused by interference with nerve impulse conduction and/or transmission. Acute DDT poisoning is characterized at the onset by marked paresthesias of the tongue, lips, and face, along with malaise and headache. In more severe poisoning, paresthesia may extend to the extremities. Ingestion of large doses is followed by vomiting with or without diarrhea. Disturbances of equilibrium, dizziness, confusion and tremor soon follow. In very severe poisoning, convulsions may appear. Blood pressure and temperature remain essentially normal. Except in the most severe cases, recovery is near complete within 24 hours. However, residual weakness has persisted for 5 or more weeks after severe intoxication. A number of deaths have followed ingestion of DDT in solvent solutions, but there appears to be no well-described fatal case of DDT poisoning uncomplicated by other pesticides or solvents.

Epidemiologic searches for chronic DDT effects have been carried out with negative results in the Northwest (Sumerford, 1953; Hayes, 1957; Hayes, 1958; Durham, 1964; Durham, 1965), the Southwest (Hartwell, 1965; Rappolt, 1968), the Southeast (Fowler, 1953; Hayes, 1955; Quinby, 1958; Ortelee, 1958; Witter, 1959; Hayes, 1965; Davies, 1966; Fiserova-Bergerova, 1967; Davies, 1968) in Hawaii (Casarett, 1968), in Alaska (Durham, 1961), and in the United States (Hayes, 1963; Hayes, 1958; Dale, 1963; Durham, 1965a; Durham, 1965b; Foter, 1966). These studies have been summarized recently (Hayes, 1967; Hayes, 1969). Human volunteer studies involving DDT are described elsewhere in this report as are epidemiological studies of workers exposed to DDT in the course of their occupation.

Endrin, chlordane, heptachlor, heptachlor epoxide and toxaphene also bring about increased excitability of the nervous system, as seen with DDT. The rapidity of onset of symptoms and signs, and the duration and severity of effects varies from one compound to another.

Organophosphates: The effects of the organophosphorus compounds are principally attributable to their ability to inactivate certain enzymes, the most important of which are the cholinesterases (See section on Enzymes).

Systemic effects.—In dosages sufficient to produce systemic manifestations, the clinical picture of organophosphorus intoxication is dependent upon the route of exposure and rate of absorption as well as upon the chemical nature of the organophosphorus compound involved. Both topical and systemic effects may occur, but frequently only the systemic effects can be observed.

Initial signs and symptoms of intoxication are headache, nausea, abdominal pain, vomiting, diarrhea, sweating, and weakness. In moderate to severe cases of poisoning, there may also be salivation, lacrimation, dyspnea, cyanosis, muscle fasciculation, convulsions, cardiac arrhythmias, shock, coma, and death. Death, when it occurs is usually due to respiratory failure.

At least one organophosphate pesticide, Isopestox (Mipafox) (bis-isopropylamido fluorophosphate) has been shown to be capable of causing demyelination of peripheral nerve fibers with resutlant neuropathy (Bidstrup, 1953). This compound has not been used in the United States. Scattered reports are available which suggest that two of the more commonly used organophosphate compounds, parathion (Petry, 1951) and malathion (Petty, 1958) may also be capable of causing peripheral europathy. The evidence cited in these reports is not convincing. Furthermore, if these two widely used compounds were, in fact, neurotoxic, it would be reasonable to expect that over the years, a multiude of cases of neuropathy would have been reported and attributed to them. That this has not been the case strongly suggests that the original allegations were incorrect.

Topical effects.—Skin—Contamination of the skin by organophosphorus pesticides may produce localized sweating, erection of hair, and fibrillation of the muscles underlying the exposed area (Hayes, 1964). For additional information on the effects of organophosphorus compounds on the skin, the reader is referred to the special report on Cutaneous Sensitization.

Eye—Contamination of the eye by organophosphorus pesticides produces miosis, loss of accommodation, headache, eye pain, dimness of vision, nausea and vomiting, conjunctival edema and hyperemia, rhinorrea and nasal congestion (Grob, 1953). When the miosis is unilateral, there is also a loss of kinetic but not static depth perception (Upholt, 1956). More detailed considerations of the effects of organophosphorus compounds on the eye are presented in section on the eye.

Pulmonary—A discussion of the local effects of organophosphorus compounds on the tracheo-bronchial tree and lungs will be found in the Section of this report on the respiratory system.

Gastro-intestinal—The local effects of organophosphorus compounds on the gastrointestinal tract have not been clearly described, probably because these effects are obscured by systemic manifestations resulting from gastroenteric absorption of the toxicant. In cases of ingestion of massive amounts of direct cholinesterase inhibitors, death or recovery with reactivation antidotes occur so quickly that clinical evidence of local effects on the gastro-intestinal tract are difficult to recognize. However, after ingestion of organophosphorus compounds which are slower enzyme inactivators such as diazinon or malathion, it may be possible to distinguish the explosive and uncontrollable diarrhea that persists during chloinesterase reactivation therapy. Such uncontrolled diarrhea often persists for some time after the other systemic signs and symptoms have been controlled by treatment. Sloughing of the intestinal mucosa has also been observed, however, this may be due to the effect of the solvent in which the pesticide is formulated rather than to direct chemical damage from organophosphorus compound (Quinby, unpublished data).

Carbamates and carbamoyl oximes: Many of the carbamates are rapid but reversible inactivators of cholinesterase and, as such, may produce serious human intoxication. These compounds may also affect the eyes and the mucous membranes of the mouth and throat topically in a manner similar to organophosphate compounds. (Babione, 1966; Quinby, unpublished data).

Field tests with Baygon (2-isopropoxyphenyl N-methylcarbamate), carried out in Nigeria and Iran resulted in overt poisoning among applicators and residents alike and indicated that clinical manifestations of intoxication correlate poorly with both red blood cell and plasma cholinesterase levels in the stricken individual (Vandekar, 1965; Vandekar, 1968). Carbaryl (1-Naphthyl N-methylcarbamate) has also caused poisoning among workers (Hayes, 1963 Clinical

Handbook). Temik (2-Methyl-2-(methylthio) propionaldhyde O-(methylcarbamoyl)oxime) caused a near fatal case of fulminating intoxication in a woman who ate the tip and several leaves of a mint plant which had grown adjacent to rose plants whose roots had been treated with Temik (Quinby, unpublished data).

Chlorinated phenoxy-acid compounds: Several authors have attributed peripheral neuropathy to exposure to 2, 4-Dichlorophenoxyacetic acid (2,4 D) (Goldstein, 1959; Fullerton, 1968). In all cases, signs and symptoms followed gross overdose resulting from exposure of the skin to the liquid compound for many hours.

Organic mercurial compounds: Organic mercurial compounds are commonly used as fungicides. Poisoning by organic mercurials is characterized by signs and symptoms of nervous system involvement such as headache; paresthesias of the tongue, lips, fingers, and toes; fine tremors of the fingers and hands; and general incoordination. Irritability and bad temper are often early manifestations of over exposure. Sever intoxication may produce total incapacitation or death.

Acute human poisoning by organic mercurial compounds has been reported infrequently but there have been many cases of chronic poisoning, most of which were associated with the manufacture of organic mercurial compounds, their use for treating seed, or the eating of treated seed (Hayes, 1963).

Effects on the nervous system.—Inorganic arsenicals: Acute inorganic arsenical poisoning produces a clinical picture involving multiple organ systems. Following ingestion of trivalent arsenic, there is a characteristic delay of from one-half to several hours. Early symptoms include a feeling of throat constriction with difficulty in swallowing. Violent abdominal pain accompanied by vomiting and profuse, watery diarrhea follow. Other manifestations of systemic involvement include muscular cramping, headache and, in severe poisoning, convulsions, coma and death, Buchanan, 1962)

Studies carried out on several hundred wine growers who were exposed for long periods to arsenical insecticides revealed electrocardiographic evidence of cardiac damage (Butzengeiger, 1949: Cited by Buchanan, 1962). In California, during the period 1951 to 1963, there were 42 fatal arsenic poisoning cases involving children. The compound most often responsible was a sodium arsenite-containing herbacide. The removal in California of this dangerous product from the home market in 1961 was accompanied by a reduction in fatal childhood arsenical poisoning. (West, Milby, 1965)

Chronic arsenical poisoning may be divided into three phases. In the first phase, the victim complains of weakness and loss of appetite. There may also be nausea and vomiting. The second phase of intoxication is characterized by symptoms of coryza, hoarseness and mild bronchitis. Perforation of the nasal septum is a common finding. Skin manifestations are common at this stage of poisoning. The third phase of chronic arsenical intoxication is marked by peripheral neuritis, which is often mild, at first, but which may progress to motor paralysis in more severe cases (Buchanan, 1962).

Thallium: Thallium sulfate is used as an insecticide and rodenticide. Human poisoning is usualy the result of ingestion by a child of thallium containing bait. Between 1954 and 1959, over 130 children in southern Texas, alone, were poisoned by thallium in this manner. (Reed, 1963)

Thallotoxicosis is characterized by polyneuronitis, epilation, gastrointestinal symptoms, encephalopathy and retrobulbar neuritis. A bluish line may appear in the gums. The gastrointestinal and neurological manifestations appear 12–14 hours after ingestion of the toxin (Grunfeld, 1964). Epilation beginns in 10 to 14 days. Persistent neurological damage was found in 54 percent of children who had recovered from thallium poisoning. (Reed, 1963)

Effects on the skin.—It has been suggested that, although pesticides are used extensively, they do not appear to produce skin disease as frequently as certain other groups of chemical agents such as household chemicals and cosmetics (Fregert, Hjorth, 1968). Since little or no relevant data are available from other states, reports published by the California Department of Public Health indicate that this may not actually be the case. These reports suggest that pesticide-induced skin conditions are more likely unrecognized or unreported than uncommon. The table summarizes reports of occupationally-related skin conditions attributed to pesticides and other agricultural chemicals which were received by the State of California during the years 1964–1968. Since equivalent data are not available from other states, the national picture can only be surmised.

For additional information, the reader is referred to the report on the effects of pesticides on the skin which may be found elsewhere in this section.

Chlorinated hydrocarbons: The chlorinated hydrocarbons do not appear to be an important producer of dermatoses among the general population. DDT has been applied directly to the skin and clothing of countless thousands of individuals as a disease vector control agent with few or no problems referable to the skin. Workers engaged in the production of lindane have been reported to suffer dermatitis as a result of exposure to an impurity, delta-heptachlorocyclohexane. (Hegyi and Stota, 1965; cited by Hjorth, 1968). Purified lindane has been used for several decades in the United States as a treatment for scabies and lice with little problem (Hjorth, 1968).

In Turkey, hexachlorobenzene-contaminated grain was ingested by several thousand people in 1956. As a result a number of cases of acquired porphyria cutanea tarda symptomatica occurred (Cam, 1959; Schmid, 1960). Severe skin manifestations including photosensitivity, bullae formation, deep scarring, permanent alopecia, and skin atrophy characterized this condition.

Organophosphorus compounds: The direct cholinesterase inhibitors (such as TEPP and Phosdrin) and the rapidly activated inhibitors (parathion, demeton, disulfoton, etc.) may cause topical effects at heavily exposed sites. These include excess sweating, erection of hair, and twitching of the muscles just beneath the site of application (Hayes, GR, 1964).

Previously existing dermatitis speeds absorption of organic phosphorus compounds and of other substances. A few of these compounds or their formulations have been established as a cause of either contact dermatitis or allergic dermatitis, TEPP (Quinby, unpublished data), malathion (Milby, 1964), and DDVP (Cronce, 1968). In each of these reports, patch tests were positive for the pesticide compound in the purest form available. In the case of malathion, however, at least the etiologic agent was not malathion itself but a reactant, diethyl fumarate (Kligman, 1967).

Parathion, like malathion, has been shown to be a strong sensitizer in man (Palmienteri, 1964). However, occupational dermatitis from parathion has not yet been recorded.

Naled caused dermatitis in 12 female chrysanthemum workers in Florida. Exposure of the workers began only two hours after spraying. Patch tests were positive in 3 out of 4 tested (Edmundson, 1967).

Dithiocarbamates: Practically all of the dithiocarbamates cause at least mild dermatitis due to primary irritation (Hayes, 1963). Ziram is extensively used and it has often been reported as the causative agent (Quinby, unpublished data).

Chlorinated carbamates: Morestan causes erythematous to bullous dermatitis in spraymen photosensitized to this insecticide because of its quinoxaline ring structure, which is common to a number of other chemicals that likewise do so (Quinby, unpublished data).

Rosaniline dyes: Gentian Violet, a mixture of Rosaniline dyes has been reported to irritate skin at low levels of exposure and to cause hemorrhages in the skin and mucous membrane in persons accidentally or occupationally exposed to high concentrations of the chemical, either as a dust or in solution (Quinby, 1968).

Chlorinated phenols: Acneiform or eczematous dermatitis has been observed widely in industries using pentachlorophenol (Hayes, 1963). Acute erythematous dermatitis was produced from contact with a mixture of a paint cleaner and pentachlorophenol. The patient excreted pentachlorophenol in his urine for over 50 days (Benvenue, 1967).

Phenolic derivatives: Sodium orthophenylphenates and other salts of that compound have caused contact and allergic dermatitis in almost 100 percent of workers sorting fruits and vegetables treated with this fungistatic chemical (Scott, 1949). Even with every reasonable precaution, a significant percentage of contact dermatitis still occurs (Quinby, unpublished data).

Nitrogen compounds: Nine cases of skin irritation by herbicides have been reported in England and Africa (Smith, 1966). Exposure of the hands to Paraquat and Diquat has caused discoloration, softening and even shedding of fingernails from the topical destruction of cells in the nail matrix (Samman and Johnston, 1969). One case of nail damage from field use, three cases from manufacturing, and four cases from formulation were reported from all over the world (Smith, 1966). Random application was followed in three workers by contact dermatitis with erythema, violaceous erythema, encrustations, bullae, edema, and exudative intertrigo (Spencer, 1966).

Captan fungicide was recently reported as a cause of occupational dermatitis from apple-spraying (Fregert, 1967).

Difolatan, a carboximide fungicide, caused 264 cases of dermatitis in orchard workers in tangerine groves of Japan in 1966 (Takamatsu, 1968). The lesions and course suggested that photosensitization played a role. Barrier creams failed to control the disease.

Chlorinated acid derivatives: Twenty-nine workers engaged in the manufacture of 2,4-D and 2,4,5-T developed either chloracne, or *porphyria cutanea tarda* (Bleiberg, *et al.*, 1964). Hyperpigmentation of sun-exposed areas was limited to the head, neck, and hands. Acneiform rash and scarring had a similar pattern. Excess growth of hair involved the lateral half of the eyebrow and the temporal half of the scalp. The 29 workers were exposed to a wide range of chemicals in this process as well as hexachlorobenzene. The authors, however, were unable to find either of these two skin diseases in patients exposed to finished formulations of 2,4-D, or 2,4,5-T.

Simple chemical compounds: Reports of dermatitis from sulphur, polysulphide, and lime sulphur mixtures are so old that it is hard to find recent citations (Shepard, 1939). Safety manuals usually refer to the transient irritation of the skin, eyes, and respiratory tract (Plunkett, 1966).

Metallic derivatives: Exfoliative dermatitis occasionally follows excessive exposure to arsenic compounds and milder dermatitis follows occupational exposure (Neal, 1941; Patty, 1962). Complete loss of hair following chronic poisoning with thallium sulfate has been re-

Reports of Occupationally Related Skin Conditions Attributed to Pesticides and Other Agricultural Chemicals, California, 1964–68

Agricultural chemical	Total	1964	1965	1966	1967	1968
Total	2, 186	470	468	452	430	336
Organic phosphate pesticides (all)	91	17	17	26	15	16
Parathion	20	2	6	6	3	3
Systox	4	1	1	1	1	
TEPP	2	1				1
Phosdrin	1				1	
Malathion	19	3	4	7	2	3
Trithion	3	2			1	
Thimet	5	3	2			
Guthion	7	1	1	2	1	2
Bidrin	1				1	
Other and unspecified	29	4	5	10	3	7
Chlorinated hydrocarbon pesticides (all)	93	21	12	22	17	21
DDT, chlordane, lindane, kelthane	57	15	8	14	7	13
Endrin, aldrin, dieldrin, toxaphene	3		2		1	
Other and unspecified	33	6	2	8	9	8
Lead and/or arsenic compounds	21	4	5	4	3	5
Herbicides	256	52	56	43	62	43
Fertilizers	143	35	35	22	29	22
Organic-mercury compounds	17	2	2	2	7	4
Fungicides, n.e.c.[1]	62	11	10	11	15	15
Phenolic compounds	101	20	16	36	15	14
Carbamates	5	1	1	1	1	1
Sulfur	[2] 77	a	a	33	21	23
Other specified agricultural chemicals	147	57	64	12	11	3
Unspecified	1, 144	242	246	235	224	197

[1] Not elsewhere classified.

[2] Sulfur included with other specified agricultural chemicals prior to 1966.

SOURCE.—State of California, Division of Labor Statistics and Research, *Doctor's First Report of Work Injury*. Statistics compiled by State of California Department of Public Health, Berkeley, Calif.

ported (Mathews, 1968). The organic mercurials cause a wide variety of dermatoses including allergic reactions according to degree and type of exposure (Patty, 1962).

Effects on the eye.—The eye may be affected by pesticides from both direct topical contamination and indirectly as a consequence of systemic poisoning. Reports from California for 1966 indicated that the eyes were involved in 27 percent of all reported occupational accidents or poisonings attributed to pesticides (California State Department of Public Health, 1966). Most of these injuries were conjunctivitis due to the irritative effects of these compounds.

Chlorinated hydrocarbons: Thermal decomposition of chlorinated hydrocarbon produces various chlorine containing compounds which are highly irritating to the eyes. Other than nonspecific irritation, there appears to be no important effect of chlorinated hydrocarbons on the eye.

Organophosphorus compounds: When eye contamination is bilateral, direct cholinesterase inhibitors cause signs and symptoms clinically identical to the optic disturbances seen in systemic poisoning. Contamination of one eye with TEPP, Phosdrin, Schradan (OMPA), Bidrin, and other direct inhibitors causes excess tearing, miosis, increased near and far accommodation, decreased light perception, and defective kinetic, but not static, depth perception. Either unilateral or bilateral miosis caused by contamination produces symptoms of mild headache, sensations of pressure in the orbit, burning of the eyelids, rhinorrhea, and improved distant vision (through camera effect).

One hundred and fourteen men poisoned in 1960 with organophosphorus compounds in California were questioned 3 years later to identify sequelae of acute poisoning (Tabershaw, 1966). Eight of these complained of continued disturbances of vision. Six of these were attributed by the patients to the acute episode. In all six cases, an etiology other than pesticides had been named by the attending physician.

In 76 necropsies on patients dying from diazinon poisoning in India, vascular pathology in the eyes, heart, brain, spinal cord, and genitourinary systems was attributed to diazinon (Limaye, 1966). However, no consideration was given to other toxicants in the formulations involved.

Bidrin was accidentally splashed into one eye of a worker when a hose ruptured. Within 24 hours, the eye constricted and did not react to light as did the other eye. The following day, both eyes were miotic suggesting systemic absorption of the toxicant. There were no sequelae reported (Gallaher, 1967).

Carbamates and carbamoyl oximes: Topical ophthalmic effects of the carbamate Baygon, appear to be clinically identical to those produced by organophosphorus compounds except that the duration of signs and symptoms is shorter (Vandekar, 1968). The rapid and spontaneous reversibility of the cholinesterase inactivation probably explains this difference.

Chlorinated aliphatics: There have been a number of individual cases and neighborhood outbreaks of moderately severe eye irritation produced by the chlorinated aliphatic fumigants in connection with their agricultural use as nematocides (Quinby, unpublished data). The three compounds known to have produced these episodes are dichloropropane, dichloropropene, and chloropicrin.

Chlorinated phenols: Three cases of monocular retrobulbar neuritis were attributed to a mixture of chemicals used for treating furniture. These mixtures contained pentachlorophenol, ortho- and para-dichlorobenzene, and DDT (Campbell, 1952). The only chemical in common was pentachlorophenol which is not known to produce neuritis (Hayes, 1963). One case of bilateral retrobulbar neuritis followed exposure to a mixture of pentachlorophenol, dieldrin, and other unstated ingredients in a mixture used for treating furniture (Jindal, 1968).

Chloro-2 phenylphenol and sodium orthophenyl phenate have caused mild conjunctivitis as well as dermatitis in apple sorters (Scott, 1949; Quinby, unpublished data). Conjunctivitis also occurs after exposure to Gentian Violet dust is in the air, but the predominant symptom is nosebleeds.

Nitrophenols: Dinitrophenolic pesticides have not been shown specifically to cause optic disease under either approved use or accidental ingestion (Hayes, 1963). However, in 1933, 2,4-dinitrophenol was advocated as an oral agent for treatment of obesity. The consequences were disastrous when cataracts appeared as delayed effects throughout much of the U.S. (Horner, 1935).

Nitrogen compounds: Although Paraquat characteristically causes injury to the lung, it also is very erosive to the eyes (Howe, 1965). As part of the local irritant action of Paraquat, the concentrated material is capable of causing eye damage. The full extent of the injury is not apparent immediately but requires 24 hours to develop. The damage is superficial, with extensive stripping of conjunctival and corneal epithelium. Provided secondary infection is avoided and adequate ophthalmologic care available, complete healing is possible within a month. A farmer lost bulbar and tarsal conjunctiva a week after accidental contamination of the eye with fluid concentrate of a Paraquat/Diquat mixture. There was also partial loss of the cornea and reactive anterior uveitis. All tissues healed between the 11th and 18th days after exposure with conjunctival adhesions complicating recovery (Cant and Lewis, 1968a). The same authors (Cant and Lewis, 1968b) reported other cases of ocular burns with permanent corneal scarring. Six milder cases of eye inflammation were reported from manufacturing and formulating Paraquat (Smith, 1966).

Sulfur, polysulphides and related compounds: Sulfur, polysulphides and related compounds used as acaricides, insecticides, and fungicides (Shepherd, 1939), have produced moderately severe conjunctivitis in formulators and spraymen (Quinby, unpublished data).

Metallic derivatives: Organic mercurial pesticides irritate the mucous membranes of the eyes. Chronic exposures of workers handling treated seeds have also produced optic neuritis with loss of irregular portions of peripheral fields of vision (Bidstrup, 1964) and atrophic changes of the fundus (Katsunuma, 1963).

Elemental pesticides: The crude lime-sulphur cooked or uncooked mixtures used as fungicides on fruits since the latter part of the 19th century (Torgeson, 1967) have caused mild to moderate dermatitis and conjunctivitis apparently from the primary irritating characteristics of either oxides of sulfur or polysulphides formed in the heating process (Quinby, unpublished data).

Vehicles: Reversible corneal injury from chemical keratitis caused by accidental contamination of the eyes with propellants in aerosol-type dispensers of pesticides has been reported (MacLean, 1967a).

Effects on the Respiratory System

Chlorinated hydrocarbons: Chlorinated hydrocarbons are not known to affect the respiratory system directly but may be absorbed through this route like most other chemicals (Hayes, 1963). Thermal decomposition products of lindane (Hayes, 1963) and of Perthane (Quinby, unpublished data) irritate the respiratory tract.

Organophosphorus compounds: Organophosphate compounds exert a profound effect upon the respiratory tract. Systemic poisoning by these toxins is accompanied by broncho-constriction and hypersecretion of bronchial fluids and in severe cases, pulmonary edema. These manifestations are a result of intense parasympathetic stimulation which characterizes the action of organophosphorus pesticides. Of even greater consequence to the act of respiration is the effect of organophosphates on the voluntary muscles of respiration, which is to interfere with transmission of nerve impulses across the neuromuscular junction. During the early phase of poisoning this interference gives rise to muscle fasciculation and weakness. In the severe case, muscle paralysis and death from respiratory insufficiency follow.

Bronchoconstriction resulting from topical application of TEPP (Tetraethyl pyrophosphate) has been reported (Quinby and Doornink, 1965).

The organophosphorus compounds have been reported by some observers to cause or exacerbate bronchial asthma (Weiner, 1961). However, other investigators have been unable to substantiate these findings (Sumerford, 1953; Hayes and Dixon, 1957a; Ganelin, 1964a; Jegier, 1965; Jegier, 1964b; Gardner, 1968; Fowler, 1953; Davignon, 1965).

Carbamates and dithiocarbamates: Both spraymen and inhabitants who breathed dust swept from dirt floors in houses treated with Baygon complained of bitter taste in mouth, and irritation of the lips, and nose and coughing for a short time after exposure (Quinby, unpublished data).

Similar but more severe signs of respiratory tract irritation is frequently seen in workers exposed to dithiocarbamate dusts such as ziram (Hayes, 1963).

Chlorinated aliphatics: The respiratory irritation caused by chloropicrin has been well-known since World War I, but its use as a nematocide in California has also caused instances of respiratory irritation so severe, that local residents had to leave their homes until the gas cleared. Dichloropropene is another nematocide which may produce the same effect (Quinby, unpublished data).

Chlorinated phenols: Pentachlorophenol causes irritation of the respiratory tract even at dosages that do not produce systemic diseases (Hayes, 1963). Dusts containing 2-chlorophenylphenate and sodium orthophenylphenate also cause nasal and bronchial irritation as well as nosebleeds (Scott, 1949; Quinby, 1968).

Nitrophenols: The nitrophenolic pesticides cause remarkably little respiratory irritation even when quite large amounts are being inhaled and the skin deeply stained. Skin staining with dinitrocresols or dinitrophenols does not mean that the worker has been poisoned. However, fatal cases have also shown staining of the lungs with edema and hemorrhages (Hayes, 1963).

Nitrogen containing pesticides: Paraquat causes fatal poisoning if swallowed in sufficiently large amounts. All deaths have been a result of proliferation of cellular elements in the lung with attendant impairment of ventilation. No systemic poisoning has resulted from its use in agriculture where only local irritant manifestations on skin, eye, or nasal mucosa have been observed.

Paraquat is absorbed poorly from the intestine and the fraction absorbed is excreted rapidly in the urine; the bulk of it is excreted unchanged within 48 hours. Because of the progressive nature of the pulmonary fibrosis, death was often 3 or more weeks after ingestion (Barnes, 1968). Individual case reports are available for more detailed description of the clinical picture of Paraquat poisoning (Howe, 1965; Clark, 1966; McKean, 1968; Anonymous, 1967; Almog, 1967; Manktelow, 1967; Matthew, 1968; Campbell, 1968).

Among the many cases that have recovered from Paraquat poisoning have been some manifesting severe hepatic and renal involvement, where steroid therapy was instituted early (Weidenbach, 1969).

Miscellaneous: Weiner and Worth (1969) reported a positive correlation between history of asthma, chronic bronchitis, and sinusitis, and exposure to household insecticides. No specific causal association has yet been established.

Effects on the cardiovascular system—Chlorinated hydrocarbons: Animals killed with large doses of chlorinated hydrocarbon insecticides show dilation of blood vessels and small hemorrhages secondary to convulsions (Hayes, 1963). Health surveys of workers with intense occupational exposure to chlorinated hydrocarbons have not detected cardiovascular changes (Laws, 1967). The possibility that elevated

DDT derived materials in body fat and the existence of hypertension may be associated has been raised (Radomski, 1968) but no definitive case for such a relationship has been established. A discussion of this observation may be found elsewhere in this report.

Organophosphorus compounds: While there is no known direct action of organophosphorus compounds on the cardiovascular system, the following changes have been observed by various investigators:

1. Hypertension or hypotension
2. Hyperglycemia or hypoglycemia
3. Bradycardia or tachycardia
4. A-V block and dissociation, exaggeration, and inversion of T-wave
5. Disappearance of P-wave
6. Cardiac arrest
7. Sub-epicardial hemorrhage
8. Acute toxic myocarditis

(Hayes, 1963 Limaye, 1966; Orlando, 1967; Comstock, 1967).

Nitrogen containing pesticides: Paraquat ingestion has been followed by myocarditis (Bullivant, 1966) and cardiac arrest (Oreopoulas, 1968).

Miscellaneous pesticides: Ingestion of elemental yellow phosphorus used as rat poison causes the usual signs of phosphorus intoxication. Symptoms of severe gastrointestinal irritation occur as soon as one-half hour after ingestion. This early stage may be followed by a latent period lasting from a few hours to a few days depending on the amount ingested. Later manifestations include abdominal pain, nausea, vomiting, hematemesis, and other hemorrhagic manifestations, jaundice, hepatomegaly, oliguria, toxic psychosis, convulsions, coma, and shock. Severe damage to heart, liver, and kidney may occur with death at any time. Cirrhosis of the liver has been reported following recovery from acute poisoning (Hayes, 1963). Electrocardiographic pattern simulates acute infarction of the anteriolateral wall of the left ventricle. X-ray confirms cardiac enlargement (Pietras, 1968).

Sodium fluoroacetate (1080) may cause death from ventricular fibrillation or cardiac arrest (Deichman and Gerarde, 1964).

Hematological effects of pesticides.—Few systematic studies of the effects of pesticides on the blood-forming organs have been conducted. Most published information consists of case reports which describe one or more patients who suffer some form of hematologic disorder and who have had recent or, in some cases, remote exposure to pesticides. While in most cases the hematological diagnosis is supported by convincing evidence, pesticide exposure factors are often poorly documented. Since hematologic disorders are not uncommon and the use of pesticides is widespread, opportunities for a chance, rather than a causal relationship must be carefully considered.

Lindane is the pesticide which has been most often implicated as a cause of hematologic disorders (West, 1967; Sanchez-Medal, 1963; Stieglitz, 1967) but other pesticides have also received attention in this regard: DDT (Sanchez-Medal, 1963); chlordane (Huguley, 1966); and parathion (AMA, 1965). However, it has been pointed out that national trends in death rates from aplastic anemia, purpura, and agranulocytosis have not changed from 1949 to 1958, a period of increasing pesticide usage (Hayes, 1961). A study in California reviewed death certificates for the period 1954 through 1963 filed in a county in which pesticides are used extensively. Comparative data were acquired from statewide statistics. No significant differences were noted between death rates from aplastic anemia, and neoplasms of the lymphatic or hemopoietic system when these two populations were compared (Rappolt 1968).

Several authors have called attention to weaknesses inherent in allegations relating pesticide exposure and hematologic disorders (Mastromatteo, 1964; Christophers, 1969; Milby, 1968). There does not appear to be sufficient evidence available at the time of this writing to categorically accept or deny this relationship.

Effects on the gastrointestinal tract.—Chlorinated hydrocarbons: It became apparent early through toxicological studies involving animals that DDT and most other chlorinated hydrocarbons could, and would, cause liver damage and dysfunction if dosage was sufficiently high and exposure sufficiently prolonged (Hayes, 1963). Actual experience, however, has not indicated that liver involvement is an important consideration in connection with human exposure to most of the chlorinated hydrocarbon pesticides. For example, Chlordane has not been found to cause detectable liver damage in cases of accidental ingestion (Curley, 1969) or in manufacturers with extensive prolonged exposure (Fishbein, 1964).

In the course of a very unusual incident, hexachlorobenzene-contaminated grain was ingested by several thousand residents of Turkey. As a result, a large outbreak of acquired porphyria cutanea tarda symptomatica occurred (Cam, 1959). The characteristic disturbances of porphyrin metabolism involved the liver (Schmid, 1960).

Over a period of 13 years, 826 workers engaged in the manufacture of chlorinated hydrocarbons were observed. No evidence of liver damage was found even in clinically poisoned men (Jager, 1968).

Organophosphorus compounds: The topical effects of organophosphorus compounds on the gastrointestinal tract have been presented elsewhere in this report.

One group of authors believed that organophosphorus compounds have a specific toxic effect upon the liver (Gitelson, 1965). They suggested that this was due to prolonged anoxia accompanying severe intoxication and not to the pesticide itself. Liver damage has not been demonstrated in recovered patients who did not suffer prolonged anoxia.

Nitrogen compounds: Esophageal casts have been vomited by patients who have ingested Paraquat formulations (O'Dwyer, 1968). Ulcers of the tongue, pharyngitis esophagitis and gastric ulcers resulted from an attempted suicide (Fennelly, 1968). Hemorrhagic esophagitis was found in another case (Duffy, 1968). In a review of fatal cases of Paraquat poisoning by ingestion, liver damage varying from cellular swelling with fatty change to necrosis was noted (Kerr, 1968).

Effects on the genito-urinary system.—Chlorinated hydrocarbon: While there is no recognized damage to the kidneys of man in uncomplicated poisoning with chlorinated hydrocarbon pesticides, there is growing evidence in experimental and wild animals that high dosage rates produce alterations in steroid mechnisms and in the reproductive cycles of certain birds. A more detailed discussion of this phenomenon is found elsewhere in this report.

Organophosphorus compounds: The effects of organophosphorus compounds on the genito-urinary system differ with dose and with the nature of the metabolic products of degradation.

Patients who have experienced profuse diaphoresis during acute poisoning may follow with a period of oliguria.

Moderate albuminuria, acetonuria, and glycosuria may be present during the late acute and early convalescent stages of poisoning (Hayes, 1963; Quinby, 1963). Paranitrophenol excreted in the urine of individuals exposed to parathion may cause mild dysuria (Hayes, 1963).

One group of workers noted generalized aminoaciduria in five of nine organophosphorus poisoning cases (Davies, 1967). In a later publication, these authors concluded that the aminoacidemia and aminoaciduria which they observed did not represent a pathological process. Present knowledge indicates that these disturbances of aminoacid excretion may be individual biochemical differences (Tocci, 1969).

Chlorophenols: Sodium chlorophenylphenate has been reported to cause mild nocturia and dysuria among workers exposed for prolonged periods. This effect was observed in apple sorters. One patient developed dysuria and nocturia after using a new mouth wash containing the same chemical used as a germicide. Complaints cleared when the mouth wash was stopped (Quinby, unpublished data).

Pesticide content of human milk.—In 1951, results of analysis of

32 samples of breast milk for DDT were reported in the United States (Laug, et al., 1951). Values ranged from 0.00 p.p.m. to 0.77 p.p.m.* with a mean concentration of 0.13 p.p.m. In none of the cases could the high values be correlated with unusual exposure of DDT. The authors concluded that "as yet clinical data are not available to assess whatever danger may be associated with the DDT stored in the fat or excreted in human milk in the quantities reported."

Additional reports have followed from the United States and a number of other countries which describe levels of DDT-derived materials, dieldrin, and hexachlorocyclohexane in human milk (West, 1964; Egan, et al, 1965; Quinby, et al, 1965; Löfroth, 1968; Curley, et al, 1969).

While the findings reported in these studies vary rather widely, it appears that presently in the United States the content of DDT-derived materials in human milk ranges from 0.05 to 0.37 p.p.m., probably averaging about 0.1–0.2 p.p.m. Because so few observations are available and because of differences in analytical techniques used by various investigators, these data are not suitable for the formulation of comparisons either by time or place. Thus, with regard to human milk, we do not know whether the level of DDT-derived materials has changed during the last 15 years or whether differences between various geographic areas exist. Precisely the same can be said for dieldrin and hexachlorocyclohexane, although both appear to be present in human milk in somewhat smaller quantities than DDT-derived materials.

Of even greater importance than our lack of knowledge regarding temporal and geographical variations in the content of organochlorines in human milk is our ignorance of the health implications of their presence. It has been estimated that the average breast-fed child ingests daily about 0.02 mg. DDT-derived materials per kilogram of body weight (Löfroth, 1969). This amounts to twice the acceptable daily intake (ADI) of DDT-derived materials recommended by the World Health Organization. The difficulty of interpreting this ADI in terms of risk to the nursing infant is reflected in the fact that neither the scientific community in the United States or elsewhere in the world has recommended that breast feeding be abandoned in favor of other methods of infant nutrition. Quite the contrary has been the case in that specific recommendations against abandonment of breast feeding have been made (Löfroth, 1969).

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*p.p.m.=Parts of DDT per million parts of whole milk.

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An appraisal of hazards to man from long-term exposure to pesticides

A large variety of chemical substances has been applied on a world wide basis in recent years for control of pests. The pests against which this defense is developed include insects and arachimids, fungi, nematodes, rodents, and herbs. The substances are used by agricultural workers to combat crop pests, by health workers to combat infectious disease vectors, and by individual householders to combat home and garden pests. Some 14 compounds or chemically allied groups of compounds constituted about half the total volume of pesticide production in 1966 while a large number of individually uncommon substances constituted the remainder. (Table 1.) Many of these materials have been developed during World War II and more recently. During the decade of the 1960's production of the 14 most common compounds, considered as a group, has been generally constant, with about half the compounds falling off moderately in volume and half increasing. The total volume of the numerous less common substances has approximately doubled as a consequence of introduction of new compounds and increased production of a number of the previously little used substances.

Table 1.—U.S. Pesticide Production, 1966

[Thousand pounds]

Calcium arsenate	2,890
Lead arsenate	7,328
Copper sulfate	103,416
Aldrin-toxaphene group	130,470
Benzene hexachloride	[1] 6,778
DDT	141,349
Methyl bromide	16,345
Methyl parathion	35,862
Parathion	19,444
Ferbam	1,379
Nabam	2,053
Zineb	4,721
2,4-Dacid	68,182
2,4,5-Tacid	15,489
Other organic pesticides	577,816

[1] 1963 production.

Among the more common group the best known and most common is DDT, and this compound has fallen off moderately in production. Another well known group consists of several related compounds, the most common of which are aldrin and dieldrin, and this group has increased moderately, approaching DDT in volume in recent years. The sharpest increase has been in parathion and methyl parathion, which have approximately tripled but are still produced in only about one-third the volume of DDT.

Acute toxicity associated with gross overexposure to most of the common compounds has been observed, and it may be assumed that all are capable of toxicity and death in sufficient dose. Susceptibilities of various species expressed as LD_{50} values, have been assembled by Hayes (23) for many pesticides and have been compared with human clinical susceptibility where this information is available.

It is clear that vastly extensive use of such toxic compounds might have effects on human health not recognizable as acute toxicity syndromes. Recognition of the true nature of such effects might be obscured either by the mildness of symptoms or by the long latent period. Much research has been reported, largely in the past 15 years, concerned with these potential effects. This paper will summarize the principal findings of this literature, will discuss the implications of time trends that have been investigated, and will attempt to identify areas where further research is needed. The findings can be conveniently presented in three general categories, namely studies of the distribution of pesticides in man's environment, studies of tissue levels of pesticides, and studies of health of exposed groups.

Pesticides in man's environment—Accidental exposure: Haynes (9) has described the known episodes of poisoning "epidemics", in which groups of people have been made ill by some common circumstance of exposure. The present paper is not concerned with single exposures, as were involved in all these epidemics, but the nature of exposure involved in these accidents is relevant to considerations of illness due to long continued exposure. In no instance did an acute poisoning episode result from minor carelessness with specified tolerances. Rather these episodes followed gross spillage of bulk pesticides. In most episodes the pesticide was spilled on food, but in one instance it was spilled on boy's pants. Poisoning resulted from contact when the pants were worn. In another instance a fungicide used for seed treatment caused poisoning when the seeds were eaten. The treatment was a preparation for planting, and it was never intended that the seeds should be eaten.

Occupational exposure: Durham and others (31) described occupational exposures in a series of job categories, ordered in terms of relative intensity of exposure. From greatest to least exposures these were (a) mixing plant personnel, (b) ground applicators, (c) pilots (of spraying planes), (d) loaders and flagmen, and (e) warehousemen.

Laws and others (*36*) classified individual workers according to the subjective rating given by their supervisors and the workers themselves as to intensity of exposure.

Some of the difficulties of such a classification become apparent in Stein and Hayes' study (*2*) of pest control operators. For this study the cooperation of the National Pest Control Association was obtained. It was learned that the average number of employees of member companies of the association was 6.2. Laws' study showed that of 1,098 former employees of one large company, in operation for 19 years, only 292 had worked for 6 months or more.

This then is in large part a group of small businesses with highly transient workers. Workers move from one type of work to another within this occupation and deal with a variety of pesticides from day to day. Recognized occupational toxicity is heavily concentrated in body surface injury, skin conditions, eye conditions, and chemical burns. Respiratory conditions are less common. Systemic poisoning accounts for a large proportion of cases, and it may be presumed that the routes of intake include skin absorption, respiratory tract absorption, and oral ingestion.

Quantitative estimates of occupational exposures have been made for DDT and dieldrin by reference to accumulated deposits of these compounds in body fat of the workers. Durham (*36*) has prepared a graph from data of various investigators indicating concentrations in fat corresponding to constant dietary intakes of DDT and dieldrin. By use of this relationship it is possible to say that a specified occupational exposure is "equivalent" to a known constant dietary intake of pesticide. In this sense Laws' high-exposure workers have an intake corresponding to a dietary intake of 18 mg. per day DDT, while his medium- and low-exposure men have intakes corresponding to 6.2 and 3.6 mg. per day, respectively. These values may be compared with an estimated 0.2 mg. per day DDT in diets of "general populations," to be discussed below. Thus some workers appear to have chronic occupational exposures almost 100 times common general population exposures.

Heavy environmental exposure: Quimby and others (*1*) have studied populations living within 500 feet and within 5,200 feet of regions undergoing agricultural application of pesticides. No actual measures of air concentration or food contamination for these people are reported, but in terms of fat concentrations no unusual intake appears to have occurred as a result of this proximity to pesticides.

General population exposure: A number of investigators have made estimates of exposures to pesticides relevant to general populations. Campbell and others (*32*) give the following estimates for the total of DDT and its most common degradation product DDE (table 2).

The estimate for food in table 2 is derived from a number of detailed studies, discussed further below. The estimates for air and water are based on very few observations and the category "Other" is largely speculative. The principal conclusion drawn by the authors is that the food component is of overwhelming importance. While this pattern of exposure probably represents the great majority of the U.S. population to a reasonable approximation, there is reason to suppose that the picture is rather different for a substantial minority. This conclusion follows from the population distribution of pesticide

TABLE 2.—*Annual intake DDT plus DDE*

	Annual intake	DDT plus DDE	
		Concentration	mg.
Air	13,000 m^3	2×10^{-4} microgm per m^3	0.03
Water	0.364 m^3	0.02 parts per billion	0.01
Food	560 kg	0.08 parts per million	44.8
Other [1]			5.0 (?)
Total			50.0 (?)

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[1] Other includes skin adsorption and intake resulting from individual household use.

concentrations in fat, to be discussed below. This distribution characteristically shows up to 10 percent of individuals with pesticide concentrations in fat higher than reasonably explained by observed distributions of these substances in diet. While some of these are probably individuals with occupational exposures not identified in the research, it must be considered that the category "Other" in table 2 may become the predominant component of pesticide intake in many instances. This could occur with habitual unregulated household use and other unusual habits of exposure practiced by a few percent of general populations.

The principal quantitative data on exposure to pesticides for general populations comes from food studies. Duggan and Weatherwax (*30*) have reported one such study and have reviewed a number of others. These studies establish the ubiquitous distribution of a wide variety of pesticides in U.S. diets. Chlorinated organic pesticides are present at detectable levels in all foods except beverages. Approximately half the consumed pesticide load is in meat, fish, poultry, and dairy products. It may be mentioned that the pesticide in these foods results from consumption of pesticide by the animals involved and not from direct application of pesticides to the animal products.

Hayes (*6*) has shown a significant difference in dietary intake for the following dietary categories: (*a*) low values in diets of "meat abstainers" (individuals who permit only certain limited use of meat in their diets), (*b*) intermediate values, in the range of 0.2 to 0.3 mg. per day of DDT plus DDE, in institutional meals of restaurants, prisons, and hospitals, and (*c*) high values, averaging 0.5 mg. per day, in household diets. DDT is present in both cow's milk and human milk, in somewhat higher concentration in human milk. Milk tests up until the date of this 1966 paper had shown no increase in DDT concentration since 1950.

Campbell and others (*2*) have assembled a number of detailed reports indicating concentrations of a variety of pesticides in a number of foods and in various parts of the United States.

Pesticides in body tissues.—An extensive literature describes the findings of many investigators who have studied pesticide levels in body tissues. The greatest part of these reports concerns studies of fat obtained at autopsy. A smaller number of biopsy samples, usually obtained at the time of surgery for a variety of operative procedures, generally agree with autopsy findings. Radomski and others (*16*) in 1968 extended this type of study to include concentrations in liver and brain, and Casarett and others (*17*) studied some 12 different tissues. Davies and others (*21*) have studied blood levels and have emphasized the value of this technique for obtaining samples from large numbers of living individuals with minimal trauma.

Hayes (*37*) has studied volunteers exposed to doses of 3.5 mg. per day and 35 mg. per day DDT. The establishment of an equilibrium level of DDT in fat has been shown to result in about 12 months on these levels of exposure, and the attained equilibrium level has been shown to increase with increased exposure. The principal degradation product of DDT is DDE, and this compound is also stored in fat. The equilibrium condition between oral intake, deposited DDT, and deposited DDE is not attained within 12 months and is the subject of continued study.

Variations of DDT concentrations in body tissue with age, sex, race, geographic location in the United States, and in a number of countries, occupational exposure, diet, and time have been studied. Davies and others (*21*) have done an extensive study of this sort, based on a variety of available populations, including autopsies, mothers undergoing Caesarean section, newborns, children, certain employee groups, and institutional inmates. The total of these populations is 509 individuals, and demographic subgroups by age, race, and sex are in many cases represented by very small numbers. Some findings of this study are the following:

> Newborn infants and cord blood have easily detectable levels of DDT and its products.
>
> Race and sex differences are not demonstrable at birth with the sizes of samples used.
>
> Levels at age 6 to 10 years are substantially higher than those of infants, but for most groups there are no substantial further increases in concentration with age 10 and higher.
>
> Levels for Negroes are substantially higher than those of whites at similar ages, same sex.
>
> Sex differences are small and inconsistent for white populations, but for Negroes male levels are higher than female.

These findings agree in general with those of other investigators.

Pesticides other than DDT and DDE have been studied in tissue deposits in several investigations, but the wealth of detail has not yet been obtained. Wasserman and others have demonstrated dieldrin in autopsy tissue from several countries (*5*). Robinson and others (*8*) failed to detect endrin or heptachlor but did demonstrate lindane in small quantities and HEOD (residue of aldrin and dieldrin) in moderate quantities in England. Casarett and others have reported levels of heptachlor and of dieldrin in a number of autopsy tissues (*17*). Dale and others (*18*) have shown levels of hexachlorcyclohexane, dieldrin, and heptachlor in populations of India. Edmundson and others (*20*) have studied variations of dieldrin by age, sex, and race, with the general observation that these variations are less than corresponding demographic differences for DDT levels. Hoffman and others have shown that in Chicago lindane is the only pesticide other than DDT found reguarly in autopsy fat (*27* and *35*). Zarvon and others (*28*) have failed to demonstrate endrin but have found lindane and heptachlor epoxide in samples from four areas within the United States.

A number of geographic comparisons, within the United States and between countries, are presented in the above material on both DDT and other compounds. Edmundson and others (*20*) have emphasized the need for caution in most such comparisons because of lack of information as to demographic characteristics of the population samples used in different studies.

One of the most important correlates of pesticide levels from the point of view of assessing general population hazard is time. Campbell and others (*32*) have presented table 3 as indicative of time trends:

TABLE 3.—*Percent distribution of DDT levels in human fat, as determined by several investigators*

Range p.p.m.	1951	1956	1958	1963
0	20	0	3	1
0.1 to 1	9	0	0	20
1 to 5	28	25	69	65
5 to 10	28	61	25	13
10 to 20	12	14	3	1
Over 20	3	0	0	0
Total	100	100	100	100

Hoffman and others (*35*) in discussing these and similar data for DDT and other compounds conclude that the storage of DDT products in human fat has not increased in the period 1951 to 1966. Davies and others (*21*) argue against accepting this conclusion in view of the lack of strict comparability of populations. It may be noted that Edmundson (*20*) and Davies (*21*) are correctly pointing out potential errors in data analysis but are not showing that the deficiencies noted have, in fact, resulted in any error in interpretation of trends.

Medical findings in relation to pesticide exposure.—To assess the hazard of pesticides to health, studies have been made of physical findings, symptoms, and laboratory findings.

Laboratory studies may serve both as measures of exposure and as measures of resulting pathology. The tissue levels of chlorinated hydrocarbons, discussed above, are generally interpreted as measures of exposure. Similarly levels of DDA in urine, the principal degradation form of DDT in urine, and paranitrophenol in urine, the major metabolite of parathion, reflect exposure and are not considered measures of pathology.

This distinction between measures of exposure and measures of effect is not always clear. Thus Casarett and others (*17*) and Hayes (*23*) have discussed the possibility that DDT levels in fat may themselves constitute a health hazard. Rapid mobilization of fat in nutritional deprivation may result in sufficiently high residual DDT levels to produce conventional toxicity. Hayes notes that this is theoretically possible, since the elimination of fat is more rapid than that of DDT. It is not possible to see such a result with stored dieldrin, since dieldrin is excreted more rapidly than fat. Such toxicity has actually been demonstrated for DDT in rats in laboratory experiments.

Decrease in plasma cholinesterase frequently serves as an indicator of exposure to organic phosphorus insecticides, but extreme depression of cholinesterase, seen in acute toxicity, is itself an emergency pathologic finding. Whether chronic mild reduction of cholinesterase is pathologic, and if so at what level, is not known.

Similar considerations are relevant to abnormal distributions of serum and urine amino acids in agricultural pesticide workers described by Davies and others (*12*), to abnormal renal phosphorus reabsorption described by Mann and others (*7*, *12*), and to stimulation of liver microsomal enzymes discussed by Radomski and others (*16*), Thompson and others (*34*), Davies and others (*33*), and Hayes (*23*). These laboratory findings are not known to be associated with any symptomatic illness. The possibility is under investigation, however, that one or another of these deviations may interfere with physiologic response to disease or with metabolism of drugs or of other toxins.

The reports to be discussed in the following paragraphs are concerned with relations between pesticide exposure and measures of morbidity and mortality. These include studies of specific diseases investigated because of suspicion raised by the toxicologic nature of the drugs or because of acute toxicity effects. Also included are studies of general diagnostic distributions, designed to identify diagnoses associated with pesticide exposure but not suggested by any prior information, and studies of such general measures as overall morbidity, overall mortality, work absenteeism, and length of hospital stay.

1. Specific morbidity conditions: (*a*) *Blood dyscrasias.*—Three pesticides, chlordane, gamma benzene hexachloride, and parathion were categorized by the American Medical Association in 1965 as known hemotoxic, with effects aplastic anemia (all 3 pesticides), thrombocytopenia, leukopenia erythroid hypoplasia (chlordane and gamma benzene hexachloride are listed as associated with the last 3 effects). Hayes (*9*) and Christophers (*13*) have questioned the evidence on which these listings are based. Brown (*19*) has received the studies reporting associations and lists an increased leukopenia in a group of apple growers (*39*), a myelogenic blood dyscrasia following exposure to benzene hexachloride, and a number of case reports of blood dyscrasias associated with lindane (gamma benzene hexachloride).

(*b*) *Neurologic abnormalities.*—Durham and others (*31*) investigated the familiar complex of central nervous system symptoms following severe poisoning by organic phosphorus compounds to determine whether a mild form of this complex was found at low levels of exposure. In a study of workers with a variety of degrees of pesticide exposure together with a group of individuals with no known exposure these authors were unable to demonstrate any correlation between mental alertness and exposure. They did repeat the finding of previous studies of a decrease in alertness as part of the symptom complex of acute poisoning, and they concluded that these symptoms were demonstrable only at exposure levels sufficient to cause other clinical signs of systemic illness. Hayes (*9*) came to a similar conclusion in a study of lapses of attention following pesticide use.

Davignon and others (*39*) studied 441 apple growers together with smaller numbers of persons living in the environment of the apple growers, but not involved in the care of the orchards. A control group of 162 people with neither occupational nor environmental exposure was studied. Neurologic abnormalities were identified both by medical history and by special neurologic physical examination. Growers showed increased objective neurologic signs, and environmentally exposed persons showed intermediate rates between growers and controls. A similar conclusion was found by relating frequency of findings to duration of exposure for the growers. Subjective neurologic symptoms were reported significantly more often in the growers and environmental contacts, with the contacts showing a somewhat higher frequency than the growers. The authors note age and sex differences among the three groups studied and suggest that a part of the neurologic differences described may be attributable to these two variables. No analysis is undertaken to take this into account.

(*c.*) *Respiratory symptoms and signs.*—One study of home use of pesticides in Hawaii showed reduced ventilatory function and higher prevalence of asthma associated with frequent application of spray insecticides.

2. General measures of morbidity and mortality.—(*a.*) Stein and others (*2*) obtained data on the entire illness history of employees of firms belonging to the National Pest Control Association and their household members, including household members who had died within 15 years prior to study. These data were obtained by means of a mailed questionnaire. The authors conclude that the data give no indication that exposure to pesticides has affected health of personnel to any significant degree.

A number of methodological problems were encountered in this report. Response was obtained from only 12 percent of member companies and from only 20 percent of employees. The survey was directed only at employees currently active at the time of survey so would not include persons who had left employment except in the case of inactive employees who were reported as members of households of active employees. Although the questionnaire asked about all present and past illness excluding colds, influenza, and childhood disease, only 14 percent of employees who returned questionnaires reported any disease at all. This is compared by the authors with the finding of 40 percent found to have disease in a health examination survey in another industry. The principal comparison as to illness is made between servicemen, clerks, and administrators. Only 7 percent of clerks reported any illness and over half of these failed to answer the question as to years of pesticide exposure. As the authors note, the clerks apparently thought the questionnaire did not apply to them. It is not clear that administrators had greatly different exposure history from servicemen. At least 83 percent of servicemen and 78 percent of administrators had worked with pesticides for over a year, and the median number of years exposed is actually greater for administrators. Thus methodology seems to invalidate any comparison with other studies, and the only available internal comparison involves two groups whose differential exposure is unknown and may be very small.

(*b*) Hayes (*9*) and Hayes and others (*37*) have described the health of volunteers who ingested DDT 3.5 mg. per day and 35 mg. per day for 21 months and were observed for another 27 months. The study has given some of the most satisfactory data available on metabolism of DDT in humans. The general health of the participants was also studied, but methodologic problems limit the usefulness of that part of the study. The authors report that no illness was observed that did not have an easily recognized cause clearly unrelated to exposure to DDT.

The study included 34 volunteers assigned to ingestion of DDT at either 3.5 mg. per day or 35 mg. per day. One of these dropped out before the first dose and two more dropped out in the first month. Only 10 completed 1 year, three on the 3.5 mg. per day schedule and seven on the 35 mg. per day schedule. Only four completed 18 months, all on the 35 mg. per day schedule. An extensive health evaluation with special reference to neurologic findings was made at the time of ending study for each individual. A variety of symptoms reported by one volunteer are attributed by the authors to "psychoneurosis," though no psychiatric evaluation is reported. It is said that changes were observed in various volunteers in weight, blood picture, and vital signs, but none was correlated consistently with increased dosage of DDT or with increased duration of exposure. Clearly with these small numbers of subjects a correlation would have to be very high indeed to become apparent, and one could hardly anticpate a statistically significant result. An extensive series of neurologic tests was done, including tests for loss of coordination and tremor. All participants remained normal in regard to these.

(*c*) Brown (*19*) reviews two additional studies of general health of heavily exposed groups. One study showed no ill effects, while the other showed only transient changes in electroencephalograms with complete recovery on removal from exposure. This latter study involved 300 workers observed over 1,300 man years' exposure. Brown concludes that there is little evidence to suggest a threat to health from current use of organiochloride pesticides.

(*d*) Laws and others (*36*) made an intensive study of 35 heavily exposed DDT production workers. Workers were categorized as high, medium, or low exposure subjects. A large number of abnormalities of physical examinations and laboratory tests are reported. These are not listed separately for the three exposure groups, though with the small total it is understandable that no such comparison was attempted. The authors note that none of several other population health studies is appropriate for comparison. Two findings are thought to represent possibly increased frequency of pathology, namely 8.6 percent diabetes mellitus (three cases) and 14 percent increased lymphocyte/granulocyte ration (five cases). The authors conclude that their findings overall do not differ significantly from those one might expect from a group of similar age and socioeconomic status without DDT exposure.

(*e*) Three studies present data from autopsy material relating pesticide levels to pathology (*16*, *17*, *35*). The principal positive finding reported by Casarett and other (*17*) was an increased level of organochloride pesticides in patients with all three of cachexia, carcinoma, and liver disease. Radomski and others (*16*) found increased pesticide concentrations in patients with any of the conditions cirrhosis of liver, carcinoma, or hypertension. The third paper (*35*) found no positive associations between pathology and pesticide level. The specific triad noted by Casarett was not investigated in this paper, though liver diseases and cancer considered separately were investigated and showed no suggestive relationship.

The authors of the two studies with positive findings carefully avoid implying a causal effect of pesticides. Radomski and others, however, are able to exclude the explanations of length of hospital stay or inanition as mechanisms for producing artifactual associations.

Discussion.—The material that has been described points to two suggestive conclusions. First, it is suggested that pesticides currently in use are responsible for little or no human morbidity or mortality other than that of acute toxic episodes due to massive exposure. Second, it is suggested that a physiologic equilibrium has been reached with the present level of exposure to DDT and that continued exposure at this level will result in no additional tissue accumulation in humans.

1. Hazards of current pesticide use.—A number of exceptions to the first conclusion have been noted. These include the following:

(*a*) Leukopenia in apple growers
(*b*) Neurologic signs and symptoms in apple growers
(*c*) Respiratory signs and symptoms in home sprayers
(*d*) Transient changes in electroencephalograms in organo-chloride pesticide workers
(*e*) Cachexia, carcinoma, liver disease, and hypertension associated with elevated pesticide tissue levels at autopsy.

In addition individual case histories of certain hematologic conditions in persons with pesticide exposure have been interpreted by some as evidence of hemotoxicity. Finally certain laboratory findings unassociated with clinical disease have been proposed as evidence of organ damage with potential delayed effects in morbidity or mortality. The paucity of conditions listed here and the general lack of confirmation among studies gives only weak support to the argument of a health hazard from occupational or general population exposure to pesticides.

A number of negative studies have also been described, and we can examine the strength of this negative evidence. Several of these involve extremely small numbers of exposed individuals. Such small samples are, of course, appropriate only for identifying extremely common conditions at the exposure levels studied. Methodologic problems have been discussed which largely negate the findings of a mailed questionnaire study (*2*). Several studies of occupational groups are concerned with the health of presently employed individuals but present no information on discontinued employees. Autopsy studies are as unsatisfactory in presenting negative evidence as with positive associations.

Hayes (*23*) has presented an excellent review of toxicological principles related to the present problem. He points out the generality of dose-response relationships and the necessity of taking advantage of these relationships in studying hazards associated with low doses. Low level DDT exposure is essentially universal, thus making unexposed controls unavailable. Nevertheless much can be determined about low level hazards from study of populations exposed at a variety of relatively high levels. As a general rule, if the incidence of some pathologic condition has been determined at some high dose, the same condition may be expected at low incidence at some lower doses, often with a longer latent period. The dose-incidence relationship may be such as to suggest a zero incidence at some finite dose, the "threshold" dose, and all lower doses. The dose-latent period relationship may imply a latent period longer than life expectancy for some low dose. Commonly a given dose is more toxic when administered over an extended time period than when administered in a single exposure. This increased effect with long-term exposure is often of the order of a doubling effect and is rarely as much as a 10-fold effect. As Hayes points out, the concept of zero incidence is of limited usefulness and must be taken in relation to the size of population under consideration. If an apparent threshold has been established in studies of 100 exposed individuals, it must be understood that a lower apparent threshold would probably be found in studies of 1,000. These general principles do not permit any precise quantitative extrapolations from high dose to low dose risks. One can, however, speculate as to the nature of dose-response relationships and the consequent low dose risk.

Hayes has given 6 mg. per kg. as the lowest DDT single oral dose with known clinical effect, while 10 per kg. is said to result in clinical effect in 50 percent of exposed persons. If the lowest dose with clinical effect has been determined in observations of populations of 100 exposed to this rather massive dose, it implies an observed incidence of at least 1 percent. It may be further supposed that the observed incidence was probably less than 10 percent, since, if as many as 10 individuals had shown a clinical effect at this dose, several would probably have shown an effect at a somewhat lower dose. Occupational exposures and experimental exposure of volunteers have involved repeated daily oral doses of 0.5 mg. per kg., or one-twelfth the lowest single oral dose with known clinical effects. Whether daily doses of 0.5 mg. per kg. continued for periods of months constitute a greater or less insult than a single oral dose of 6 mg. per kg. is not clear, but it seems reasonable that these may represent similar orders of magnitude. These heavily exposed workers and volunteers were therefore probably at risk of 1 to 10 percent clinical effects, and with the small sizes of populations studied it is not surprising that no effect was demonstrated.

Lightly exposed workers and some individual heavy pesticide users probably experience lower exposure by a factor of 10, and present day populations with only dietary and incidental exposure experience lower exposures by a factor of 100 (i.e. 0.005 mg. per kg. per day or 0.35 mg. per day for an average sized man). If we imagine linear dose response curves with no threshold, the incidences of clinical effects would be 0.1 to 1 percent for lightly exposed workers and 0.1 to 1 per 1,000 for present day populations. The available negative studies generally involve populations too small to identify incidences in this range. Long latent periods may occur at low doses, and most present studies are inappropriate for investigating such effects. It has been noted that studies of active workers, without followup of discontinued workers, will systematically tend to miss any serious condition whose prodromes influence continuing employment. The mild neurologic symptoms (*39*), respiratory symptoms (*4*), and "psychoneurosis" (*37*) noted in various studies could result in such a selective bias.

If only trivial diseases are involved, incidences of the order of 0.1 to 1 percent in occupational exposure and 0.1 to 1 per 1,000 in general populations can probably be accepted as commensurate with the beneficial results of pesticides. If, however, serious conditions with obligatory latent periods, such as malignancies requiring a cell multiplication interval for recognition, are involved, these risks may not be acceptable. Other late appearing conditions that may be considered are neurologic degenerative conditions and chronic obstructive respiratory disease. The possibility of malignancies is suggested by correlations found in autopsy material (*16, 17*) and by laboratory animal studies (*38*). It should be emphasized that the authors of all these reports have cautioned against a causal interpretation in human malignancies but rather have indicated an area in which positive findings remain unexplained.

2. Time trends of hazards.—The principal data relative to time trends relate to tissue levels of DDT, and the general finding is a constant level over recent years or a moderate fall in level. Methodologic difficulties with this evidence have been discussed and relate to lack of comparability of survey procedures at different periods of time. The difficulties might work either to exaggerate or to obscure time trends. There is no evidence specifically suggesting that increasing hazards have been overlooked.

A similar conclusion comes from observations of DDT levels in foods being consumed by humans.

Durham (*14*) has suggested that a similar steady state has been reached for dieldrin. For other pesticides time studies are unavailable or inadequate for judging trends.

Summary.—A review of published reports of epidemiological studies of human hazards from long exposure to pesticides fails to demonstrate any definite increase in morbidity or mortality attributable to these compounds. Exposure is found to be essentially universal throughout developed and "developing" countries of the world, over all social classes, over ages from newborn to all adult ages, and for both sexes. Wide variations of exposure exist, with extremely high levels being associated with occupational exposures.

Published data similarly demonstrate no increase in exposure in recent years to the most commonly used pesticides.

While the two above conclusions argue against a present or imminent hazard, a number of deficiencies in the available evidence have been cited. Studies are not adequate for recognizing conditions of low incidence, of the order of 0.1 to 1 per 1,000 in a short interval following exposure. Studies are not adequate for identifying diseases first appearing after latent periods of several years.

There is no clear evidence linking human malignancy causally with pesticides. Associations of malignancy with pesticide level in autopsy material, however, remain unexplained. Carcinogenesis has been demonstrated in experimental animals, rodents and fish, from pesticide doses that have no counterpart in human exposure.

The following types of studies are recommended in relation to the above observations:

1. Continued study of pesticide levels in foods for human consumption. Standard methods for food sampling and for pesticide analysis for the common compounds should be established in order to make interpretation of time trends possible.
2. Pesticide levels in human tissues should be studied in population samples selected to represent general populations. The availability of techniques for analysis of pesticide levels in blood makes such studies feasible. Standard sampling and analytic methods should be adopted for time trend analysis.
3. Epidemiologic studies appropriate for identifying low incidence conditions should be carried out.
4. Epidemiologic studies appropriate for identifying conditions appearing after latent periods of several years should be carried out.
5. Longitudinal studies of heavily exposed populations should be carried out. Populations to be investigated may include: (a) Workers with high and medium occupational exposure, (b)

persons who have experienced one or more acute toxicity episodes, (c) persons with very high tissue pesticide levels not apparently related to occupational exposure.

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SPECIALISTS REPORTS ON POTENTIAL HEALTH EFFECTS

Cutaneous aspects

Allergic contact dermatitis refers to an inflammatory process of the skin mediated by an as yet uncharacterized antibody which adheres to circulating white blood cells. This implies that on first contact with a pesticide there will be no reaction. There then ensues an incubation period of approximately 1 week, at which time the next exposure leads to a dermatitis. Not everyone contacting the pesticide will react; only those with antibodies. A common example of this phenomenon is poison oak-poison ivy dermatitis.

Extent of the problem.—Inherent in diagnosing allergic contact dermatitis is the method of proving this scientifically. Inasmuch as the antibody has not yet been characterized, the only available method is the patch test. This consists of application of a low chemical concentration to the skin under adhesive tape occlusion to increase penetration. If an individual has antibody, the disease is reproduced in miniature under the pitch in 24+ hours.

Patch testing is generally done when an allergen is suspect. It is legend in the field of occupational dermatitis that a diagnosis cannot be made until an allergen is suspected and a proper knowledge of patch testing is available. Unfortunately, there is very little knowledge available on proper methods of patch testing with pesticides. Many physicians are unwilling to do this for fear that the amount of material under an occluded patch test may produce systemic toxicity. Many agricultural workers attempt to make their own diagnoses and struggle with their disease. There are only a few centers in the United States extensively involved in diagnostic patch testing. Of these, none have specialized in developing methods that could be recommended for use by the general physician.

Published literature.—Hjorth and Wilkinson recently reviewed contact sensitization to pesticides (*1*). It was their general opinion, from the minimal information available, that dermatitis from pesticides is uncommon. They reviewed largely single case reports. Perhaps the most meaningful were those of Fraegert. This author is one of the few dermatologists in the Western world working full time on occupational dermatitis. It is unlikely that any problems he finds exist only in the area of Sweden in which he works. It is more likely that he is trained, has the time and energy, and is thus able to make specific diagnoses. He demonstrated an allergic dermatitis in agricultural workers to several pesticides, including Rodannitrobenzene, Captan, Phaltan, and an ingredient of 4-chloro-2-methyl-phenoxyacetic acid (*2–4*). The latter pesticide was very important insofar as technique is concerned; the diagnosis would have been missed by routine patch testing for he reacted only to an intermediary product and not to the commercially available material.

Edmundson and Davies reported a small epidemic due to Naled in 9 out of 12 workers in a chrysanthemum farm (*5*). Spencer documented allergic contact sensitization to 2-chloro-N,-diallyl acetamide in 3 farmers (*6*).

Milby and Epstein had the opportunity of surveying a population of California agricultural workers exposed to Malathion and quickly demonstrated sensitization in 4 out of 157 (3 percent). The most important outcome of this study was that sensitization was not due to the compound they tested (Malathion), but to an intermediary product—diethyl fumarate. The practical significance was that when this information came to light the manufacturer decreased the amount of this intermediary product present in Malathion which then presumably decreased the incidence of future sensitization (*7, 8*).

Recently, Takamatsu et el., surveyed an outbreak of dermatitis in tangerine orchards in Japan due to a new fungicide (Difolatan) which sensitized 25 percent of involved workers in 1966, and 38 percent in 1967 (*9*). This incidence of sensitization as of significance, in terms of the suffering and costs involved.

Interpretation.—At the moment, no hard figures are available to determine the extent of the allergic contact sensitization problem in man. From the experiences of Milby and Epstein, Fraegert, Spencer, and Takamatsu et al., we suspect that sensitization is probably much more common than is generally realized.

Recommendation.—Basic science information on allergic contact dermatitis is, of course, a great help. But more specifically, certain areas of information are urgently required for which technology is available and costs are minimal. First priority would be given to defining proper patch test concentrations so this information could be made readily available to all physicians involved in diagnosing occupational and agricultural dermatitis. Only when this is accomplished can proper diagnoses be made and valuable epidemiological data become available.

Secondly, we need relative information on allergic sensitization potential of commonly used pesticides. This can be accomplished in the guinea pig and rabbit using currently available modifications of the Draize-Landsteiner techniques. (*10*). It would also be advisable to verify this information in terms of ranking the severity of sensitization potential in human volunteer testing, using the well-established human Draize test (*11*).

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Substantivity

Substantivity is a term derived from classical 19th Century dye chemistry. It concerns the mordant effect of a dye. In cutaneous physiology it pertains to a chemical's ability to adhere to skin, no matter what the physical or chemical explanation.

Extent of the problem.—If a compound is rapidly washed off the skin by workers, there should be less chance of percutaneous penetration, and therefore less risk of toxicity. If the materials are not readily removed, opportunity for penetration (and toxicity) increases.

Literature.—The only pertinent study of which we are aware is that of Fredrikkson who studied the decontamination of human skin with Parathion (*1*). Using P^{32} labeled material he demonstrated that a soap and water wash for 30 seconds (which is far longer than most workers would wash) removed only 36 to 48 percent of the applied dose, if the wash was delayed for 6 hours. An alcohol wash (in which Parathion is soluble) still allowed 10 percent of the dose to remain.

Interpretation.—The substantivity of pesticides for human skin is of great practical importance in terms of long term toxicity.

Recommendation.—Methods such as those used by Fredrikkson and some more recently elaborated by others, allow a practical system to determine the substantivity of pesticides. Data should be obtained on standard pesticides, not only measuring the degree of substantivity but demonstrating fool-proof and practical methods of removing these chemicals by workers. The technology is available, the risks to human volunteers extremely minimal, and the decrease of potential toxicity great. Obtaining this information clearly deserves a high priority.

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Cutaneous metabolism

Extent of the problem.—Human skin is, contrary to older beliefs, an active metabolic organ. This not only includes viable cells in the epidermis and dermis, but also the presumably dead stratum corneum. There are numerous enzymes in this stratum corneum that can hydrolyze many chemicals, including DNA and RNA.

Implicit in the study of animal toxicology is the fact that skin exposure allows the penetration of chemicals analogous to that which is injected into animals. If the materials are first hydrolyzed and absorbed as different materials, the animal toxicology may not be relevant. We know that Parathion is not hydrolyzed on animal or human skin, but this is the only pesticide for which we have such information (*1*).

Interpretation.—Cutaneous metabolism, apart from that of other organs, is an area of great potential significance.

Recommendation.—With the ready availability of radio-labeled materials, it is recommended that the ability of skin to metabolize commonly used pesticides be obtained. If these products of hydrolysis are indeed different, then animal toxicology should be obtained on them.

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Percutaneous penetration

Percutaneous penetration refers to the amount of a chemical that gets from the skin's surface into its various components and is eventually absorbed into the systemic circulation; it is then available either to be excreted in urine and stools or stored in an organ such as fat (i.e. as with DDT).

Extent of the problem.—Classical pharmacology has not often included studies of percutaneous penetration. This science has involved itself mainly with the effect of drugs, usually taken either orally or parenterally. In the case of pesticides, exposure is more likely to be cutaneous than parenteral. Although respiratory exposure is of importance, investigators such as Wolfe, Armstrong, and Durham have clearly demonstrated quantitatively that exposure is far more cutaneous than respiratory (*1*, *2*). For instance, they demonstrated that when Parathion concentrate is used with spray equipment the respiratory exposure is less than 0.1 mg./hr., whereas cutaneous contamination is 27 mg./hr. They demonstrated examples such as air blast spraying equipment where cutaneous exposure reached over 750 mg./hr. These doses must clearly be considered in the range of drugs.

It has classically been assumed that cutaneous barriers are so complete that few chemicals can penetrate, and that penetration is only of significance if one deals with the most potent of chemicals, such as the war gasses. Recent data has clearly demonstrated that the cutaneous barrier is far from complete, and that many chemicals pass through the skin with ease (*3*).

Techniques of observations.—Until recently, human experimentation involving percutaneous penetration was rarely done. Classical experiments included comparison of the LD–50 in the skin as compared to parenteral administration. This information is valuable as far as acute toxicity was concerned, but of little value in determining body exposure in long-term usage. Fredrikkson studied the penetration of Parathion and Paraoxon *in vitro* (*4*). This involved use of a small glass chamber in which chemical was applied on the skin and the material studied as it came through the dermal side. He compared this data to that of percutaneous penetration measured by the surface disappearance technique. Here, a radioisotope detection system is used on the surface of the skin and its disappearance noted. It is assumed that as the counts decrease the material is being absorbed. It should be noted that this technique can only be used for very hard rays as weaker rays are unable to reach the counter. He noted there was far less penetration in the *in vitro* chambers than the *in vivo*, utilizing the disappearance technique.

Nabb, Stein, and Hayes (W. J.) studied the dermal absorption of Parathion and Paraoxon in rabbits and found penetration rates of 0.059 μgm./minute/cm.^{2} and 0.3 μgm./minute/cm.^{2}, respectively (*5*). When Hayes (G. R.), Funckes, and Hartwell studied the percutaneous penetration of Parathion in man using urinary excretion of P-nitrophenol, they noted as much as 23 mg. of P-nitrophenol in urine per day (*6*). Recently, Milby, Feldmann, and Maibach undertook a systematic study of percutaneous penetration in man in which radiolabeled chemical was applied to the skin and its penetration quantitated by the appearance of radiolabel in urine (and, in some instances, feces) (*7*). The advantage of this system is that when radiolabeled compounds are available one need not be concerned about developing methods to isolate each metabolite but can study the radiolabel itself. This enabled the authors to rapidly screen a series of compounds. As a requisite control to this method, a tracer dose is injected intravenously to determine how much compound is excreted in the urine or stool. Knowing how long the material remains in the human body allows one to determine the risks or more properly interpret the classical animal data. In other words, if the compound is rapidly excreted there should be less toxicity than if it is stored or slowly excreted, all other things being equal.

They showed a wide divergence in the penetration of the first pesticides studied. Dieldrin, Parathion, and Malathion all penetrated in significant amounts but were only moderate penetrants. Their penetration rates ranged from 6 to 8 percent of the applied dose (4.0 μgm./cm.^{2}). However, Carbaryl was virtually a complete penetrant in that almost 75 percent of the same dose was accounted for in the urine. Needless to say, interpreting LD–50 data becomes far more meaningful when such penetration data is available. If 1 percent of the dose penetrates as compared to 75 percent, this will greatly alter this interpretation.

These studies also showed that penetration through the skin of this and many other compounds is extremely slow. One can identify the material in urine for at least 5 days after a single application. This long-term passage has profound importance in terms of chronic toxicity of man.

Effect of delivery vehicles on penetration.—Very little work has been done in this regard. It is generally assumed that vehicles will significantly increase or decrease the penetration of certain molecules. A specific example is the study of McDermot, Finkbeiner, and Wills and Heggie in which they quantitated the effect of DMSO on the percutaneous penetration of Seman (pin-acetyl-methyl-phosphonofluoridate) (*8*). They noted that DMSO increased the potency ratio (LD–50) almost 6 times on normal skin and 4 times on stripped skin. Brown recorded significant changes in LD–50 of carbamates with varying solvents (*9*).

Interpretation.—Percutaneous penetration of pesticides is obviously a critical area in terms of chronic toxicity to man. This has only been minimally investigated because appropriate techniques have only recently become available. It is likely, from the very slow and delayed penetration through the skin and the very significant amounts of chemical penetrating the skin that this is an area of crucial importance in terms of man.

Recommendations.—As a minimum, we should have quantitative penetration data on the various commonly used pesticides. At the moment, human studies with radio-labeled materials appear the most practical for quickly obtaining data of direct relevance (*11, 12*). Other methods should also be developed to see if they are relevant to the human situation. This includes ranking of known common pesticides, using various *in vitro* systems, and other animal systems. Although we are concerned about the acute poisoning of humans through the skin (such as recently summarized by Eitzman and Wolfson who demonstrated seven of 30 deaths as clearly being due to skin contact), we are more concerned about the insidious long-term effects which this route of exposure allows (*10*).

Examination of varying vehicle systems should be considered in an attempt to decrease the skin penetration of these pesticides.

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Effect on other cutaneous organs

It is known that certain pesticides affect other keratin-producing structures in skin, such as the nails and hair. Samman and Johnston have shown that Paraquat and Diquat will produce abnormal fingernails (*1*). Presumably the mechanism of action is penetration through the nail-plate with a direct effect on the nail matrix.

Haustein (*2*) claimed he observed dystrophic human hair follicles after improper use of Fekema ES-30-9 (DDT and gamma-HCC8). This has not yet been substantiated.

Although our knowledge in this area is too minimal to allow informed interpretation, these observations suggest the hair and nail should be more closely looked at than previously in regard to an index of toxicity. Certainly, the animal hair follicle has proved an extremely sensitive index of x-radiation damage. The same information should be sought in man for pesticides.

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SAMPLE SKIN MATRIX

	Parathion	Malathion	Dieldrin	Carbaryl
Percutaneous penetration:				
Animal	Moderate penetrant	—	—	—
Man	Moderate penetrant	Moderate	Moderate	Moderate
Substantivity	Very substantive	—	—	—
Contact sensitization	Possible minimal risk	Probable problem	—	—
Metabolism	Not hydrolyzed on skin	—	—	—
Nails dystrophy	Not known	—	—	—
Hair dystrophy	Not known	—	—	—

Note.—Dashes (—) indicate inadequate information available.

Behavorial effects of pesticides

The word "behavior" has a number of meanings in common parlance and several idiomatic applications in different branches of medicine and science. These include the meanings "to carry, conduct, comfort, manage, bear, discipline, handle, restrain, regulate, act, conduct oneself, or to act in relation to the environment." One of these is the so-called "behavioral" effects of pesticides. Unfortunately, there have been some misunderstandings and misapplications of the meaning usually applied in this context. In its strictly literal sense, "behavioral effects" of pesticides are any influences the chemicals exert on the nervous system or body that alter normal or usual physiological responses in an environment containing pesticide. However, almost all pesticides in sufficient dosage are capable of causing such alterations in response. These are the usual signs and symptoms of acute or chronic poisoning. The term "behavioral toxicity" as applied in clinical toxicology has generally excluded the established signs and symptoms especially during the acute phase of poisoning. Conversely, many investigators have at first regarded as "behavioral" one or more unusual effects of pesticides not then known to be expected signs or symptoms. The subsequent better and more complete understanding of the actions of such a particular pesticide has often moved such behavioral effects over into the area of established signs and symptoms. For example, in the late 1940's, airplane pilots began to complain of disturbances of vision (depth perception and night blindness) during application of the then newer pesticides. These unusual signs were often reported in the absence of other evidences of toxicity Accidents and fatal crashes were blamed on these unusual effects then regarded as behavioral.

More than 5 years of study (Upholt, 1956) established that the responsible pesticides were the direct cholinesterase inhibitors (mostly TEPP). The effect was caused by topical exposure of one or both eyes without extensive exposure otherwise. Bilateral miosis caused night blindness. Unilateral miosis and paralysis of muscles of accommodation from unilateral exposure caused disturbances of kinetic depth perception. As these local or topical effects became more widely recognized, most toxicologists no longer regard these as behavioral effects but rather as logical expected effects of acute topical exposure. The inclusion of unusual or poorly understood effects of pesticides in the group of behavioral effects will continue to plague and confuse those concerned with health hazards of pesticides whether they are officials, authors, or the public. Unfortunately, in some instances pesticides are blamed for such effects when the causal relationship has not been well established. An example of this is the blame placed on 2,4,D as a cause of peripheral neuritis (Goldstein et al., 1959). For convenience of arrangement, the clinical syndrome of peripheral neuritis without other behavior change is presented in the section on "Effects on the Nervous System."

Understanding of the area considered as behavioral effects also requires an understanding of the difference between topical or local poisoning, and systemic or body-wide poisoning. Acute and chronic poisoning has always been best known as systemic poisoning wherein there has been sufficient absorption and circulation of the toxicant to cause the signs and symptoms of poisoning expected from the known modes of action of the pesticide. On the other hand, pesticides, like other chemicals or drugs, may act in a site-specific point in the body usually because the exposure has been limited to that part of the anatomy, such as the eye, the nose, the mouth, or a restricted area of skin. In too many instances, the local effects of a pesticide have been assumed to be the more hazardous systemic poisoning with consequent overconcern and overprecautious reduction of the exposure.

Undoubtedly the most obvious and yet the most problematic behavioral effects are the ones effecting the brain and nervous system. Our behavior is influenced most by our nervous system (with the possible exception of the reproductive system in active adults). Rarely in the general population, but more frequently in agricultural workers, applicators, formulators, and others exposed to pesticides, there have been many reports of abnormalities of behavior in the absence of other usual signs or symptoms of pesticide intoxication.

In summary, "behavioral effects" of pesticides are those behavioral signs or symptoms other than the usual and expected effects seen in either acute or chronic poisoning. They may be premonitory effects, sequellae, or after effects suspected to be, but not necessarily directly causally related to the pesticide exposure. Unfortunately, because of the sound grounds for technical disagreement over the status of any one finding or allegation of a behavioral effect, the area of discussion known as "behavioral effects" has been a hodgepodge of data, conflicting reports, and emotional publications. Some workers have avoided the field of behavioral effects, calling it a "never-never land." Nevertheless, careful consideration and study of the behavioral effects of pesticides has elucidated many health problems posed by pesticides and should continue to do so in the future. False allegations of magical deviltry of pesticides are discredited, and sound suspicions of unappreciated effects are confirmed as expected effects when the mode of action has been established. Appropriate precautions and regulatory actions can then be taken.

The term "behavioral toxicity" was first used at the conference on the evaluation of pharmacotherapy in mental illness in the fall of 1956 by Brody (1959) to describe the adverse effects of drugs on psychological functioning in animals. Although the concept of behavioral toxicity arose from Brody's study of objective responses in the operant situation, any broad consideration of behavioral toxicity in man must take into account both the subjective mood changes and the objective performance changes induced by drugs.

Clinical behavioral toxicity.—The most impressive characteristic of the psychiatric drug literature is the absence of serious concern about adverse effects these drugs may have upon behavior (Uhr H., Miller J. C. 1960).

The Ciba Foundation convened a symposium on the neurological basis of behavior in 1957. The early definitions and histories of behavior were reviewed and 18 other papers were presented on specialty aspects of behavioral studies in man and experimental animals. The last of these papers concerned the relevance of some neurophysiological data to behavior disorders. Such idiomatic terms as "consciousness," "awareness," and "personality changes" were defined and discussed in the psychiatric sense. Mainly this symposium was produced by physiologists, anatomists, neurologists, pharmacologists, psychiatrists, endocrinologists, and biologists. Toxicologists were conspicuous by their absence. A number of techniques were presented on measuring "behavior" objectively (Wolstenholme, 1958).

A number of good books have been written on the behavioral effects of pesticides as reflected by the expected effects of acute poisoning on the nervous system by chlorinated insecticides (Von Oettinger, 1955) and by anticholinesterase compounds (Koelle, 1963; Heath, 1961, and O'Brien 1960, 1967).

The chlorinated hydrocarbon insecticides, especially DDT, have been known to act in the cerebellum, brainstem, spinal cord, and peripheral nerves (Bromiley and Bard, 1949; Shankland, 1964). Thus the acute effects of these compounds appear to be scattered widely throughout the nervous system. Moreover, they have topical action on the nerve endings in the mucous membrane (Hayes, 1963) and may extend their action proximally on the nerve pathways as shown by various case reports. For over 20 years, toxicologists have studied pesticides mainly by investigating morbidity, mortality, growth, pathology, and storage in experimental animals and man. Much less attention has been given to the possible behavioral effects of dosages below those required to effect gross clinical signs and yet sufficient to possibly alter the behavior of man and animals. Studies on the effects of pesticides on sensitive indicators of brain function have not received appropriate attention (Ruffin, 1963) and almost as little attention have been given to psychobiology.

A group of industrial toxicologists in Holland followed 826 workers in the manufacture of chlorinated hydrocarbons (aldrin, dieldrin, telodrin) for 13½ years. They found no behavioral effects past the "temporary signs and symptoms of specific intoxication (Hoogendam, 1962; Hoogendam, 1965; Jager, 1968).

Toxicologists in Russia have naturally fallen under the influence of Pavlovian behavioral physiological training (Ruffin, 1963). These Russian workers are trained differently and reason differently than do American psychologists, physiologists, and toxicologists. One of these Russian toxicologists has extensively reviewed the application of studies of conditioned reflexes of experimental animals in response to the more important groups of pesticides (Medved, 1964). They have established definite changes in behavior of animals far below levels required to produce symptoms or at levels of cholinesterase activity just under the preexposure range. Changes were also noted in reflexes before they could detect functional or chemical changes in their liver and carbohydrate metabolism. Both of the usually reversible changes studied as well as totally irreversible changes such as caused typically by organic mercurial pesticides.

Behavior changes in workers manufacturing DDT and related chemicals have already been mentioned under another section of this report (General Effects: Clinical Effects from Case Reports) by another Russian but translation difficulties limit interpretation of that report (Paramonchik, 1969). This latter industrial toxicologist was convinced he observed "the asthemic syndrome and autonomic dystonia" in some workers. Unfortunately these people were also exposed admittedly to many other chemicals as well—not given in their exposure histories—and no mention was made of the workers' habitual consumption of alcohol that may account for some symptoms.

As early as 1962, one American toxicologist stressed the importance of mechanics of "toxic stress" in industrial toxicology and environmental pollution. However, as of 1969, far too few studies are in progress in this country attempting to measure behavior and other effects below that sufficient to cause frank, acute toxic signs (Stokinger, 1962). Over a decade ago a behaviorist conclusively showed that rats respond to stressful agents producing radical changes in behavior (Richter, 1958). The center in the brain suspected of reflecting this disturbance was the hypothalamus.

The altered locomotion patterns of rats fed as little as 100 p.p.m. of DDT was demonstrated by study of track patterns in charcoal-dusted runways (Khairy, 1959).

Conditioned reflexes in cats treated with aldrin were changed from the eighth to the 13th day after starting daily oral dosage with only 1 mg./kg. of aldrin. Changes returned to normal responses thereafter but sometimes the restoration of normal function was cyclic in pattern (Spynu, 1964).

Stumptail monkeys showed altered behavior at dosages considered no greater than some people ingest daily for a period of weeks. The treated group showed depression of arousal and emotional levels. There were other observations of lessened response to environmental conditions (Thomsen, 1969).

In another study of adolescent stumptail monkeys (*Macaca speciosa*) orally treated with low dosages of endrin, search was made for subclinical or clinical subacute effects based upon biochemical, physiological, hematological, pathological, histological, and behavioral changes. In general, few changes were detected when there was no toxic patterns in individuals or groups. Results indicated that only the differential white blood cell count could be used as a valid indicator for toxicity in future pesticide research (Barth, 1967).

An experimental carbamate pesticide closely related to several now in growing use in this country such as carbaryl and Baygon diminished the self-protective responses of rats (Goldberg, 1963). The rats had previously been trained to avoid shock by pushing a lever as a response to a light. Prior to treatment with the carbamates the rats were able to respond with 95 percent or better; efficiency decreased to ast low as 50 percent 30 minutes after the administration of the carbamate at a dosage of 1 mg./kg. intraperitoneally. Those doses were considerably lower than those which produce symptoms of anticholinesterase activity. A comparison of the ED 50 with the LD 50 revealed a ratio of about 1 to 25. However, the cholinesterase inhibition in both the brain and the erythrocytes paralleled the behavioral changes. Thus there appears to be no behavioral changes with these compounds without cholinesterase inhibition. In fact, dose-response studies indicate that the threshold effects on discrete avoidance studies are associated with about a 50 percent inhibition of brain cholinesterase.

Without more closely controlled data on human exposure, more consideration must be given to pertinent experimental toxicology in animals. A neurochemical approach was made with a study of brain chemistry in rats exposed to carbaryl (Hassan, in press). In acute and chronic dosage producing no signs or altered behavior, levels of brain norepinephrine, serotonin, and 5-hydroxy-3-indolylacetic acid increased but dopamine did not. This means that biosynthesis is increased, certain biogenic amines are released and catabolism is increased (without signs detected). Thus, some biochemical basis for behavioral changes in rats can be detected before the changes themselves.

Studies on altered behavior due to anticholinesterase effects received more research attention than those due to chlorinated hydrocarbon pesticides. One of the best reviews by a Russian worker (Medved, 1964) in English presented extensive information on the effects of organophosphorus insecticides on conditioned reflexes of animals. Changes in these reflexes appear to be evident only after detectable cholinesterase inhibition. A return to normal reflexes occurs after prolonged low-grade exposure to the cholinesterase inhibitors. This indicates adjustment of the central nervous system to the increased content of acetylcholine. Thus, the biphasic effects of chlorinated hydrocarbon insecticides as an initial change in function, followed by a return to a normal state despite continuing action of the chemical under test, is also apparent with the anticholinesterase compounds.

Without tests of subclinical activities of pesticides, the capability of clinical toxicologists to resolve maximum-allowable-concentration controversies or to safety establish maximum daily intake allowance has been aptly questioned (Ruffin, 1963).

Using mainly clinical and laboratory toxicologic methods including electroencephalography, Dutch industrial toxicologists surveyed 826 insecticide workers in mainly chlorinated hydrocarbons without finding more than temporary signs and symptoms of acute toxicity (Jager, 1968; Hoogendam, 1962).

An Italian group of workers (Giachetti and colleagues, 1966) reported that male rats treated with 0.24 mg./kg. of parathion on alternate days for 1 month do not develop blood cholinesterase inhibition but do exhibit a slight inhibition of brain cholinesterase and the *learning* of a conditioned avoidance reflex was hindered. At this low level of dosage, there was no interference with the conditioned reflex once it was learned. Thus it appears that the learning of a conditioned reflex may be a very sensitive indicator of the functional effects of exposure to cholinesterase inhibitors. This decrease in learning capability obviously should receive further study.

Other American workers (Grob, 1953; Holmes, 1964; Holmes, 1965; Bowers, 1964) reported similar effects on the behavioral changes in man following anticholinesterase administration. The syndrome observed has been broadly classified as a state of altered awareness, fatigue, increased irritability, difficulties in coordination, slowed mental processes, forgetfulness, muscular aches and pains, malaise, and lack of self-control. Some of these effects have been observed over a year after last exposure.

One of the military toxicologists tested only a classified nerve organophosphorus agent capable of rapid skin absorption (Bowers, 1964). By applying minute doses of the war agent to the skin, he produced marked behavioral changes as the blood cholinesterase level fell more often before gastrointestinal symptoms set in than afterwards. At these low dosage levels, there occurred no muscular, optic, pulmonary, or lower bowel signs or symptoms as should be seen in classic organophosphorus poisoning. Sometimes the behavioral changes occurred several hours before two other classic expected gastrointestinal signs occurred. In a significant fraction of the volunteers, only behavioral signs appeared when cholinesterase levels went as low as 10 percent of preexposure level. Thus, no correlation of psychologic and gastrointestinal symptoms were found. Behavior changes usually did not occur until the whole blood cholinesterase fell to 40 percent of control or lower. Atropine treatment improved mental function as had been found by prior workers (Grob, 1947).

The military toxicologist attributed the behavioral syndrome to a slight excess of physiologically active acetylcholine in the central nervous system (Bowers, 1964). People concerned about the validity of these behavioral effects, which are disputed by most toxicologists, should be aware of a deficiency of logic on the part of one of the originators of the contention that behavioral effects occur during and after exposure to organophosphorus compound (Holmes, 1964; Holmes, 1965). Just because symptoms disappear after treatment with atropine does *not* mean they were due to concurrent exposure to organophosphorus compounds. Something else is more likely to be causing these symptoms if they are not typical of organophosphorus poisoning and accompanied by significant cholinesterase inactivation. For example, a pesticide mixer went to his doctor in 1954 with "symptoms typical of a severe cold which proved on further examination to be due to anticholinesterase insecticide poisoning (Holmes, 1964). The symptoms disappeared promptly after atropine therapy." The symptoms of a cold should always be benefited by treatment with atropine whether the man was exposed *or not*. Just because he was exposed did not make the exposure the cause of the symptoms. If the worker had other symptoms clinically suggesting organophosphorus poisoning, they should have been presented in sufficient detail to be convincing. Until such convincing data becomes available, the contentions of their observer must always be considered as reflecting some ungrounded bias. He overlooked the greater probability of a cold instead of organophosphorus poisoning. This worker also studied 24 patients receiving an organophosphorus drug, echothiophate iodide, as eyedrops daily for glaucoma. Of these 24, 84 percent experienced, within 8 months, pronounced red cell cholinesterase inactivation as great as the pesticide workers' acute exposures. Although nine patients developed symptoms, six did not. Of these with symptoms, none must have been definite behavioral changes or the author would have made a special point of them. Moreover, the distributor of echothiophate has followed the use of this drug for at least 7 years and has not detected behavioral changes such as have been alleged to pesticides (Anonymous, 1969).

In addition to functional disturbances, neuronal lesions of the nervous system were recognized early as caused by a pesticide by a British toxicologist. Mipafox produced classic signs for polyneuritis in three manufacturers in 1951 but this pesticide has *never been used* in the United States of America. (Bidstrup, 1953). Even earlier, the same pathology had been recognized in far earlier manufacture of a related nonpesticide phosphate (TOCP) (Hunter, 1944).

Following the earliest publication of recognition of subclinical and postclinical or postintoxication behavioral effects produced by organophosphorus compounds in a limited percentage of those exposed, many clinicians in many countries began to report all sorts of minor behavioral deviations all the way up to complete psychosis. In 1958, two cases of mixed-type damage to the nervous system were attributed to mixtures of pesticides (Petty, 1958). The first case was exposed to parathion, EPN, DDT, dieldrin, and lead arsenate; the second to DDT and malathion. Although there is no doubting, the neurologic lesions produced, the exposure history and the clinical courses were not at all indicative that pesticides were responsible. Febrile illnesses and drug treatments also complicated the interpretation.

Two Australian workers surveyed in 1961 a fruit-growing area extensively using organophosphorus pesticides and found 16 cases of psychiatric sequelae they attributed to pesticides (Gershon, 1961). They compared no control population. The type of psychotic sequelae were mainly depressive and schizoid tendencies but practically all the other behavioral symptoms were detected in the exposed population as they should have also been measured in comparable populations. Perhaps some, but not likely all of these cases should be attributed to organophosphorus compounds. The compounds suspected were malathion, guthion, parathion, trithion, and others.

This study, in part, triggered an epidemiological investigation in Australia to determine psychiatric sequelae—especially schizophrenia and depressive states—of exposure to organophosphorus compounds. The results revealed no such relationship (Stoller, 1965).

Another nonpesticide phosphate drug (DFP) caused reactivation of florid psychotic attacks in patients with this prior history (Rowntree, 1950). These psychotic changes persisted for some months. On the other hand, schizophrenics were improved by the intravenous injections of acetylcholine (Fiamberti, 1946). In a rebuttal to the above Australian's allegations that schizoid and depressive states were caused by organophosphorus poisoning, a critique by an English authority pointed out that if eight of 10 greenhouse workers in one greenhouse had psychiatric changes from pesticides, then the world experience should have produced psychotic tendencies in 80 percent of people with comparable exposures. Such an exorbitant incidence would have been obvious in all areas of extensive use of organophosphorus compounds such as fruit growing areas. This means there is something wrong with or peculiar to the Australian situation not attributable to pesticides at least at this rate (Barnes, 1961).

Federal aviation workers in 1964 contended that chronic exposures of pilots to organophosphorus insecticides were followed with anxiety, uneasiness, giddiness, insomnia, somnambulism, lassitude, drowsiness, tinnitus, nystagmus, dizziness, pyrexia, paralysis, paresthesias, polyneuritis, mental confusion, emotional lability, depression with weeping, schizophrenic reaction, dissociation, fugue, inability to get along with family and friends, and poor work performance.

Another group of public health toxicologists searched very closely from 1960 through 1962 and less intensively from 1954 to date (1969) in the Northwest without finding confirmation of the so-called behavioral effects of pesticides (Durham, 1964 and subsequent unpublished studies). Tests of mental alertness were not influenced by exposure to organophosphorus poisoning unless symptoms of poisoning appeared accompanied by cholinesterase levels low enough to explain the symptoms. One very dramatic mild poisoning case did illustrate the principle that it is very likely for a poisoned patient to have such mild clinical signs and symptoms that he will not report them to his physician or employer unless he notices a more drastic change in his own behavior such as driving his car carelessly across a sidewalk in a very dangerous place. Pilots have reported similar incidents to explain erratic behavior causing crashes.

The most important conclusion from this paper is that there have occurred changes in behavior in workers that threaten the life of themselves and others even though *they do not realize it and are not aware that they are poisoned.* Consequently, it is most likely these behavioral changes are being missed frequently under usual conditions of exposure not being closely surveyed. It is also likely that similar behavioral changes occur at least temporarily at lowered blood cholinesterase levels without other detectable clinical findings. This has been clearly established in experimental animals many times cited elsewhere and is similar to reports from chronically exposed workers (Holmes, 1965; Dille, 1964; Quinby, 1958).

An Egyptian studied 25 cases of spraymen's poisoning with Metaisosystox and appropriate matched controls. After the acute symptoms of organophosphorus poisoning had subsided, headache, dizziness, and muscular weakness, especially in the eyes, persisted for "varying periods" (Hegazy, 1965).

Canadian health workers surveyed for 3 years 441 apple orchard workers whose occupations involved occupational exposure to organophosphorus compounds, chlorinated hydrocarbons, and other pesticides considered usual for spraying fruit and other work in sprayed orchards. About 26 percent of the people exposed for from 1 to 14 years developed one or more signs and symptoms of acute poisoning in the 3-year period. Unfortunately, no distinction was made between topical and systemic poisoning. A total of 170 people living in the sprayed environment and 162 people living out of the sprayed environment were followed as controls. These workers found no signs of prolonged effects from pesticides after the cessation of acute symptoms in an unstated number of poisonings (presumably some less than 26 percent of the cases with symptoms) (Davignon, 1965).

Clinical toxicologists and epidemiologists have made a number of smaller epidemiologic surveys searching for behavioral effects attributable to heavy exposures to various pesticides, especially organophosphorus compounds and chlorinated hydrocarbons. These have all been reported as essentially negative in Mississippi (Fowler, 1953; Quinby, 1958), in Arizona (Ganelin, 1964), in Washington (Summerford, 1953; Hayes, 1957).

Two occupational health specialists retrospectively surveyed 235 persons reported by physicians as poisoned by organophosphates in California in 1960. Of the 235 reported, 114 were considered to have had organophosphorous poisoning; six everely, 54 moderately, and 54 mildly. Followup was conducted for 3 or more years. Of these, 43 had complaints that persisted up to 6 months and 33 still had complaints after 3 years. These complaints were: optic, gastrointestinal, headache, cardiorespiratory, neuropsychiatric, and miscellaneous. There were no psychotics. Ten individuals had persistent symptoms primarily referable to the central nervous system. None felt it was caused by organophosphorus poisoning. There were other likely explainable causes for six of these 10. Intolerance of odor of pesticides was mentioned by 20 (17 percent) of the poisoned patients and was believed to be psychogenic conditioning. The authors believed they would have detected serious sequelae of high incidence but would likely have missed minor after effects or major one of low incidence (Tabershaw, 1966).

The Colorado encephalographers examined sleep patterns of two people with histories of varying degrees of organophosphate exposure. Teenage and occupation-matched controls were compared. Exposed workers had REM (rapid eye movement) times not significantly different on the first night from controls. However, the exposed group showed a high proportion of unusually long REM periods, six out of 44 periods exceeding 50 minutes and three of these six prolonged periods were longer than 1 hour. In the control group, one REM period exceeded 50 minutes out of 34 REM periods. One exposed subject showed unambiguous REM periods of 107 minutes duration. While the authors present this in such a way that considerable significance might be attached to the differences, only the one prolonged REM period of 107 minutes differs from the longest control. No details of history are given to explain this difference (Stoyva, 1968).

In an earlier paper, the same group found nine of 12 subjects with narcoleptic sleep patterns and two with disturbances of normal cycling of sleep stages with unusually long stage I sleep. If these are the same 12 patients as reported in their later paper, the other unexposed controls had almost as long REM periods as all the exposed with the one patient excepted. The paper also listed a long list of psychiatric differences between the exposed and unexposed controls. The differences cited are open to statistical question.

There have been two earlier reviews of sequellae of organophosphorus poisoning. In 1965, the first reviewer from California classified the neurologic sequellae that were known or suspected to occur after poisoning with organophosphorus compounds: (1) Immediate muscular weakness persisting long after the disappearance of other signs and symptoms; (2) irreversible paralysis with demyelination; and (3) psychiatric disturbances. The one British report of Mipafox causing peripheral neuritis was accepted as completely beyond question but that reviewer concluded there was found no convincing evidence to causally relating organophosphorus exposure to psychiatric disorders in the United States (Milby, 1965).

Dr. Irma West of the California State Department of Public Health had followed occupational pesticide poisoning in that State for 14 years. She considered that brain anoxia from respiratory failure during acute poisoning as the most easily identifiable sequella of the nervous system and this has been recognized as a residual in cases with severe cyanosis. Overtreatment with atropine and hyperthemia was pointed out as another likelihood but has not been reported as such. Phenothiozine tranquilizers have increased the severity of poisoning and might add to brain damage but this has been reported only once (Arterberry, 1962). No clear-cut cases of behavior changes were reported from California in this paper (West, 1968).

For 1 to 3 months each during 2 years, four WHO toxicologists and staffs closely followed teams of sprayers, in El Salvador, Nigeria, and Iran who were treating houses for malaria mosquito control with the carbamate Baygon (Babione, 1965; Babione, 1966; Davies, 1967; Quinby, 1967; Vandekar, 1965; Vandekar, 1966). They detected up to 100 percent attack rates of acute mild anticholinesterase poisoning in spraymen and about 1 percent in the inhabitants. However, no sequellae or behavioral effects were detected beyond the 2 days of acute poisoning in each case. No epidemiologic search was possible on a systematic basis on the inhabitants living in treated homes. However, the reporting of complaints was routinized and some homes were treated three times in three cycles about 100 days apart closely observed. The investigators in El Salvador noted that new spraymen were poisoned more quickly than employees without prior repeated exposures to both DDT and Baygon.

A practitioner, coroner, and public health toxicologist noted marked behavioral changes in a mentally defective man poisoned with phosdrin and/or parathion (Arterberry, 1962). On the ninth day of recovery, he was given a phenothiazine tranquilizer for mental unmanageability. His anticholinergic signs and symptoms recurred so severely that he died. Naturally, this led to the assumption that there must be an unappreciated potentiation of organophosphorus compounds by phenothiazine derivatives. When suggested, this was later confirmed in animals by laboratory coworkers (Gaines, 1962).

Two internists attributed two cases of polyneuropathy to aldrin and endrin respectively. However, their grounds for belief for causal relationships are not convincing (Jenkins, 1964).

A pediatric toxiocologist in a poison-control center conducted detailed psychometric-psychologic evaluations on 41 children 18 months to 14 years after an acute central nervous system poisoning. No overall differences were seen in these children when compared to control patients who had ingested other toxic agents not suspected to affect the nervous system. The eight children who had had convulsions only from their intoxication, however, showed decreased learning ability and seven of the eight had behavioral difficulties. Chlorinated hydrocarbon pesticides were the principal cause of convulsions in this group of eight children (Angle, 1968).

Behavioral effects of solvents.—Kerosene or xylene and related distillates in the air can cause dizziness, poor coordination, and confusion leading to coma if the patient does not get better ventilation (Hayes, 1963). Some victims have been mistakenly believed to be drunk on alcohol until the proper history was obtained.

Undiluted DMSO produced a depression of spontaneous motor activity without an effect on hexobarbital sleeping time or the conditioned avoidance response of rats (Weiss, 1967).

Behavioral tests.—The Continuous Performance Test measured the ability of a subject to react to different 0.92-second sequences of letters within 0.69 seconds (Rosvold, 1956).

Seconal and chlorpromazine will act to disturb the cortical activating system of the brain and produce impairment in performance on the Continuous Performance Test (Mirsky, et al., 1959).

Nonfocal (centroencephalic) patients perform more poorly than focal epileptics on the test of attention, whereas, there was no difference on the memory test (Mirsky, et al., 1960).

Sleep loss caused decreased performance on the Continuous Performance Test (Kornetsky, 1959).

Both sleep deprivation and chlorpromazine produced marked impairment in the performance of the attention test, less so with the former—all subjects showed slowing of the electroencephalogram, increased respiratory length and increased finger pulse amplitude as compared with performance of subjects when in normal state (Mirsky, 1962). A hypothesis was presented to explain the dissociation of the effects of various control acting drugs and other agents (Ed note: Such as pesticides) on performance on the Continuous Performance Test and the Digital Symbol Substitution Test. The tests were affected differently because they depended on a functioning of different neurological organizations in the brain (Mirsky, 1964).

In the pharmacological context of behavioral effects of drugs, there should be no questioning that organophosphorus compounds, whether they be drugs or pesticides, do have behavioral effects changing the response of the patient to dosages of neurotropic chemicals sufficient to produce them. Psychosis, reactivated by DFP (Rowntree, 1950), persisted for months after the dosage stopped. On the other hand, schizophrenics improved with injection of acetylcholine (Fiamberti, 1946). As in dosage response to drugs, there is a great range of dosage response levels in patients to pesticides. Subjective mood changes have been carefully measured for drugs but not as well for pesticides. Conditions of observation for pesticides have not been as well controlled except in experimental animals where conditioned reflexes and other behavior changes have been well documented. Many clinicians have described all degrees of behavioral changes from brief inattentiveness to violent mania in acute organophosphorus poisoning. Some observers have found these less severe behavioral changes in exposed patients without other classic signs of poisoning.

Confounding the causal relationships of pesticides to behavioral effects are the many other undisputed causes of behavior changes:

1. Highly prevalent psychoneuroses and psychoses of nonpesticide origin. This includes the toxiphobias and emotional antipathies to pesticides that are indirectly caused by pesticides. Direct causal effects

have been established of pesticides causing psychogenic odor, abhorence, and even nausea with vomiting. Unfortunately, too many epidemiologic studies of behavior changes from pesticides have not included adequate control populations to measure all these nonpesticide-caused behavioral changes.

2. Sleep deprivation and its severe effect on behavior is insufficiently appreciated by investigators and the public. Many pesticide workers endure sleep deprivation during and after exposure.

3. The influence of alcohol upon behavior is well-known but difficult, if not impossible, to measure in conditions of occupational or population study.

4. Drugs have been proven to interact and potentiate acute poisoning but studies below obvious poisoning levels in humans are unreported if not undone.

5. Financial motivations:

Money changes human behavior in relation to pesticides. Medical insurance payments, rewarding or loss litigation, and industrial compensation have caused patients to malinger and industries to minimize or conceal toxic effects.

6. Other miscellaneous causes of behavior changes such as aging, trauma, and hormonal changes in behavior have been documented before the patient was aware that he was poisoned and sought medical diagnosis and care.

Needless to say, a great deal of more careful study needs to be done on behavioral effects of pesticides on humans and in experimental animals in order to define which should be attributed solely to pesticides, to interacting causes, and to nonpesticide causes.

Conclusions.—There are obvious behavioral effects of organophosphorus pesticides during and after the acute and chronic clinical poisoning periods. There are conflicting reports of the extension of these postpoisoning symptoms for up to 2 years or longer. In experimental animals, there are behavioral changes long before onset of other classic signs of poisoning, but there is only suggestive conflicting evidence that this has occurred in man from pesticides. The proof is already available from special nerve-type chemical war agents. As with other mental aberrations, there is indisputable proof that behavioral changes dangerous to the patient and the public occur before he realizes he is poisoned with pesticides and seeks medical care. There is no doubting the damage to the nervous system up to 2 years caused by one fluorine-containing organic phosphorus pesticide (mipafox) but this pesticide has not been used in the U.S.A. Regrettably, but as expected the literature is confused by an assortment of unconfirmed allegations that organic phosphorus and chlorinated hydrocarbon pesticides cause bizarre changes in behavior that presently make no sense at all to clinical toxicologists, psychiatrists, clinical psychologists, epidemiologists, and public health physicians. Pesticides *per se* have not been established as a cause of schizophrenia or depressive psychoses even though preexisting attacks may be exacerbated by organophosphorus compounds certainly at clinically toxic levels it not below. Chemical war agents *are* known to do so, however.

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Experimental animals

The toxicity of pesticides has been studied in a variety of experimental animals in an attempt to learn about the toxicity of these compounds to man.

A correlation has been shown to exist between acute toxicity levels as determined in experimental animals and the occupational hazard of pesticides to man based on actual use experience (Gaines, 1960). Gaines has also presented evidence that there is a closer relationship between acute dermal LD_{50} values and the occurrence of occupational poisoning than between oral LD_{50} values and occupational poisoning. The prediction of occupational poisoning for man may be improved somewhat by studying the effect of repeated dermal doses of a pesticide in animals (Hayes, 1960).

There also appears to be a correlation between toxicity values from experimental animals and the occurrence of fatal poisoning in man. Hayes and Pirkle investigated the 119 deaths in the United States in 1961 which were attributable to pesticides. Six of these deaths were caused by chlorinated hydrocarbon insecticides, 24 were due to organic phosphorus pesticides, and 58 were caused by inorganic and botanical compounds.

These proportions are in general conformity with the relative acute oral LD_{50} values for these materials. In general the acute toxicity of the organic phosphorus pesticides is greater than that of the chlorinated hydrocarbon compounds; however, the persistence of the latter is greater. In the fatal cases, only 17 (16 percent) were occupational while 80 cases (72 percent) involved oral ingestion.

It is difficult to obtain data to confirm that the expected relationship exists between the toxicity of repeated doses of a pesticide in experimental animals and the hazard of repeated low-level exposures to the same chemical in man for the newer synthetic pesticides, since most of these compounds have not produced poisoning in man at low exposure levels.

The selection of an animal species to provide indications of hazard to man is important. There are some manifestations of toxicity which apparently can only be produced in certain species. For example, the delayed neurotoxicity ("ginger paralysis" or "jake-leg") produced in man by triorthocresyl phosphate and certain organic phosphorus insecticides, could be induced only in chickens and calves among various experimental animals tested.

Some toxic effects seem to have great quantitative variations between species, such as the liver histopathology seen with low dosages of DDT in rodents, but only with much higher dosages, if at all, in other species. There are also differences in the metabolic pathways of foreign chemicals, including pesticides, between different species. For example, the rat and man convert DDT to DDE while the monkey seems to lack this capacity.

In addition to studies in experimental animals, some interesting work has also been done on pesticide toxicity in tissue culture (Gabliks, 1965). This technique has been little used to date but may have potentiality as an easily standardized and controlled indicator of toxicity to animals and man.

Acute and chronic toxicity.—The most basic information on pesticide chemicals is the acute toxicity (usually in the form of the LD_{50} value) to an experimental species (usually the rat). These studies are very widely done by manufacturers, consulting laboratories, universities, and Government agencies. An up-to-date publication on the subject which lists LD_{50} values for many of the currently important pesticide chemicals has recently appeared (Gaines, 1969). The LD_{50} values given in this paper should be useful in providing estimates of relative toxicity of these compounds since all the tests were carried out under standardized conditions using the same rat stains.

Also obtained from these studies of acute toxicity is information on the symptomatology produced. In general, experience has shown that symptomatology for poisoning by a given compound tends to be similar in different species. Thus, this information is useful for man.

The principal, if not the exclusive, acute action of DDT and the other chlorinated hydrocarbon pesticides is on the nervous system. The exact nature of the action is unknown.

In animals, the earliest apparent effect of DDT poisoning is abnormal susceptibility to fear, with violent reaction to stimuli that normally would be unnoticed (Hayes, 1965). There is definite motor unrest and an increased frequency of spontaneous movements. A fine tremor appears and becomes constant, interfering with normal activity. As the nervous system involvement progresses, there are attacks of epileptiform tonoclonic convulsions. Death may result from ventricular fibrillation.

In addition to their major effect on the neuromuscular system, DDT and the other chlorinated hydrocarbon pesticides produce minor changes in the liver, especially in rodents (see later discussion) and, to a still lesser degree, in other organs.

These compounds are cumulative, being stored in body fat. Illness in experimental animals caused by repeated exposure to DDT is essentially identical with illness caused by a single dose of sufficient size.

Effective therapy is forthwith apparently restricted to efforts to remove the poison and to control tremor, convulsions, and other central nervous system effects. Short-acting barbiturates are used to control central nervous system hyperactivity. A very high dosage—enough to produce anesthesia under normal conditions—may be required and is tolerated without undue depression in the presence of poisoning. Although these various aspects of treatment were worked out in studies with experimental animals, they appear to be applicable in man.

The organic phosphorus pesticides appear to act primarily, if not entirely, through inhibition of the enzyme, cholinesterase. Symptomatology is associated with over stimulation of the parasympathetic nervous system. Differences in activity levels of cholinesterase in various species presents somewhat of a complication in making comparative studies.

However, suitable modifications can be made in the cholinesterase methodology (for example, Frawley *et al.*, 1955) to compensate for these differences.

Atropine and the oximes are antidotal in both experimental animals and man.

In pesticide toxicity studies with experimental animals, a number of factors have been found to be important in determining the response of the animal to the toxicant. These factors include differences in the physiological state of the animal (species, age, sex, disease, and nutrition) and differences in the environment (temperature and light). Some of these factors have also been noted to have similar effects on pesticide toxicity in man. In other instances, data on the importance of these influences for human toxicity is not available.

1. Species.—The toxicity of pesticides, in common with that of many other poisons, varies considerably between phyla, classes, and species of animals. The high toxicity of the organochlorine insecticides generally to fish (Doudoroff et al., 1953) and the refractoriness of goats to DDT (Hayes, 1959) are examples. Rotenone is highly toxic to fish (Gersdorff, 1930), moderately toxic to certain mammals, such as the guinea pig (Shimkin and Anderson, 1936), but only slightly toxic to birds, especially the chicken (Cutkomp, 1943). Recently, it has been shown that norbormide is a rather specific toxicant for the Norway rat (Roszkowski, 1965) and it is used as a rat poison. From a practical standpoint, insects and mammals show a tremendous difference in their susceptibility to various pesticidal compounds. At least for DDT, this is due primarily to the great difference in the ability of the two groups to absorb the compound (Hayes, 1959).

Murphy and DuBois (1957) noted differences between the guinea pig, cat, and mouse in the metabolism of the active anticholinesterase agent formed from azinphosmethyl (Guthion). They suspected that the rapid destruction of this metabolite by guinea pig liver might be partly responsible for the relative resistance of this species to poisoning by azinphosmethyl.

Brodie and Maickel (1962) have studied the mechanisms by which different varieties of animals dispose of lipid-soluble foreign compounds. They were able generally to account for the relative effect of the drugs tested on various species of animals on the basis of differences in liver microsomal enzyme activity. They also made some fascinating reconstructions of the evolution of biochemical processes in the metabolism of foreign compounds. They noted that, as in many other biological processes, the development of the drug-metabolizing enzyme system in a maturing animal goes through successive stages similar to those in the phylogenetic succession.

There are both qualitative and quantitative differences between species with regard to pesticide metabolism. Thus, although monkeys (Durham et al., 1963) and rats (Hayes et al., manuscript in preparation) fed DDE store the compound in adipose tissue, and although DDE is found in the fat of rats fed DDT, monkeys on DDT diets store little or no DDE. It appears that the failure of monkeys fed DDT to store DDE is not due to an inability to absorb and to store DDE but rather to an inability to convert DDT to DDE. The monkey does excrete DDA in the urine, as do men and rats. The sex differences for DDT and DDE storage (Durham et al., 1956) and for susceptibility to parathion (DuBois et al., 1949) and to some other organic phosphorus pesticides (Gaines, 1960) that occur in rats have not been noted in other species studied. However, not enough is known at the present time about the response in man to either DDT or parathion to know whether or not a sex difference exists.

2. Age.—Newborn animals of several species have an almost complete lack of ability to metabolize certain drugs (Fouts and Adamson, 1959). However, this capacity increases quite rapidly during the first few weeks of life.

The lack of microsomal activity in the neonatal liver correlates well with the observed sensitivity of the newborn of several species to some pesticides. Thus, the toxicity of EPN (Murphy and DuBois, 1958), and of ethyl fenthion (DuBois and Puchala, 1961), was found to be much higher in 23-day-old male rats than in adult rats of the same sex.

In a very complete study, Brodeur and DuBois (1963) compared the acute oral toxicity of 16 anticholinesterase insecticides to weanling and to adult male rats. All of the phosphorothioates and phosphorodithioates tested were more toxic to weanlings than to adults. The greatest age differences were seen with EPN and carbophenothion (Trithion) to which weanlings were about five and four times, respectively, more susceptible than adult males. Weanling rats were only slightly more susceptible than adults to mevinphos, trichlorofon, and carbaryl. DEF (Folex) was about twice as toxic to weanlings as it was to adults. On the contrary, a phosphoroamide, schradan (OMPA), differed from all of the other compounds tested in that adult males were more susceptible than weanlings. The factor of difference was about five.

Lu et al. (1965) have studied the comparative oral toxicity of malathion, DDT, and dieldrin to rats of different ages. They found the toxicity of malathion increased in the following order: adult, preweaning, newborn. However, for both DDT and dieldrin, adult rats were more susceptible than newborn rats. With the exception of the newborn rats, only small differences were noted between the other age groups tested. The toxicity of DDT increased in the following order:

newborn, preweaning, weanling, middle-aged, young adult. For dieldrin the order was newborn, adult, preweaning.

The greater susceptibility of the young observed in experimental animal studies with pesticides seems also to be applicable in man. In instances in which parathion-contaminated food has been eaten by people of different ages, death has occurred mainly or exclusively among children (Kanagaratnam et al., 1960). Children 5 to 6 years old were killed by eating an estimated 2 mg. of parathion, a dosage of about 0.1 mg./kg. In contrast, daily doses of 7.2 mg. (about 0.1 mg./kg.) given to adult volunteers for a period of 42 days produced no signs of poisoning and no symptoms other than a moderate decrease in blood cholinesterase level (Edson, 1957).

3. Sex.—The acute oral toxicity to white rats of DDT does not appear to be significantly influenced by the sex of the animals tested. The LD_{50} values are 113 mg./kg. for males and 118 mg./kg. for females (Gaines, 1960). However, female rats are somewhat more susceptible to repeated doses of the insecticide than are male rats (Fitzhugh and Nelson, 1947; Haag et al., 1948). Female rats also store more DDT in their fat than male rats fed at the same dosage level (Hayes, 1959). Sex differences are apparent in the effect of DDT on liver cell morphology and in fat storage of the compound and its metabolite DDE. Male rats show much more frequent and extensive histological changes in the liver than female rats when both sexes are exposed repeatedly at moderate dosage levels (Ortega et al., 1956).

The evidence for or against a sex difference in DDT storage for other species is not so clear. However, in studies on pigs (Harris et al., 1953) and monkeys (Durham et al., 1963), no sex differences with respect to DDT storage were noted.

In the rat, a species in which both storage and toxicity frequently show sex differences, these differences may be modified by sex hormones. Durham et al. (1956) studied the influence of hormones and of gonadectomy on the storage of DDT and DDE in the rat. Testosterone propionate or oophorectomy decreased DDT storage in female rats while diethylstilbestrol or testectomy increased DDT storage in male rats. The effects on DDE storage were similar but of lesser magnitude.

In in vitro studies, Murphy and Dubois (1958) showed that the conversion of azinphosmethyl and of EPN to active anticholinesterase agents was two to three times greater in the livers of adult males than of adult female rats. No sex difference in enzyme activity was noted for animals less than 30 days of age. The low enzyme activity in the livers of adult female and of young male rats was increased by testosterone. The high enzyme activity in the livers of adult males was decreased by castration and by progesterone of diethylstilbestrol.

Administration of a male sex hormone to female rats and of a female sex hormone to male rats tended to equalize their susceptibility to a single dose of parathion (DuBois et al., 1949).

DuBois and Puchala (1961) demonstrated that ethyl fenthion had a high toxicity to female rats, male and female mice, and male guinea pigs, while male rats were much more resistant. There was no sex difference in the toxicity of the oxygen analogue to rats.

In comparison of LD_{50} values for male and female rats of 44 pesticides, the male rat was more resistant in 22 cases (50 percent), the female rat was more resistant to five compounds (11 percent), and the sexes were about equally susceptible to 17 compounds (39 percent) (Durham, 1967). There were greater differences between the sexes in susceptibility to poisoning for the organophosphorus than for the organochlorine compounds.

There appears to be little or no data on relative susceptibility to poisoning by pesticides for men and women. However, some data is available on sex differences in DDT storage levels for man.

Conclusions regarding the effect of sex on the tissue storage level in man of DDT and other organochlorine pesticides have been different in various studies of the matter. In study of 254 human subjects in Israel, Wassermann et al. (1965) did not find significant differences in storage of DDT or DDE in relation to sex. Laug et al. (1951), and Read and McKinley (1961), found that sex had no significant effect on storage level of DDT or its metabolites which were studied. However, Zavon et al. (1965) noted a tendency toward a somewhat higher concentration of dieldrin, o,p′-DDT, p,p′-DDT, DDE, and heptachlor epoxide in men than in women, although the differences were not conclusive. Robinson et al. (1965) reported higher concentrations of DDT, DDE, and dieldrin in adipose tissues from males as compared to females. On the other hand, Hayes et al. (1965) found that females stored significantly higher levels of p,p′-DDT and of beta-BHC than did males. Application of a recently developed procedure has indicated that concentrations of several organochlorine pesticides occur at higher levels in blood from males than from females in the general population (Dale et al. 1966).

4. Disease.—Diseased animals have been shown, under certain conditions, to have different responses to the added stress of exposure to a pesticide than do healthy animals.

The interaction of DDT and trichinosis has been studied in rats (Hayes and White, manuscript in preparation). Trichinosis was chosen for investigation because it was easily transmitted but presented minimal danger of unintentional spread and because, with selected dosages, it produced a disease which, although severe, was generally nonfatal in the absence of other stress. The administration of repeated doses of DDT, known to produce extensive storage of the compound in fat, increased mortality in rats infested with Trichina only slightly. Infestation with Trichina larvae caused a dramatic loss of weight which was similar in rats which received DDT and in those which did not. The changes in storage of DDT and DDE which were seen in rats infested with Trichina could apparently be accounted for by the weight loss which occurred.

Under ordinary conditions, methoxychlor has a low order of toxicity for rats and shows little tendency for accumulation in the fat and other tissues. However, in rats whose livers had been severely damaged by carbon tetrachloride, the toxicity of methoxychlor was increased markedly as was its propensity for storage in body fat (Laug and Kunze, 1951). However, in an acute study there was little difference in DDT toxicity noted between normal rats and rats with carbon tetrochloride-induced liver damage (Judah, 1949). One might interpret these data as indicating that, under these circumstances, the liver—and perhaps the liver microsomal enzymes—were of considerable importance in the metabolism of methoxychlor but of less consequence for the handling of DDT. However, Judah (1949) did note that rats and rabbits with extensive, long-standing liver damage were more susceptible to DDT poisoning than were control animals. Partially hepatectomized rats showed a somewhat greater susceptibility to dermally applied dieldrin (LD_{50}, 50 mg./kg.) than did control animals (LD_{50}, 90 mg./kg.) (Durham and Hayes manuscript in preparation).

There is also some information available on the effect of disease on susceptibility to pesticide poisoning in man.

An individual who was said to be "sickly" and hungry at the time of eating the compound, became ill following the ingestion of 6 mg./kg. of DDT (Hsieh, 1954). In other persons in this same incident and in other reports from different situations, illness followed ingestion of doses of 10 mg./kg. or greater, but smaller doses generally have not produced poisoning (Hayes, 1955).

Maier-Bode (1960 found no essential difference in storage of DDT or DDE between 21 persons who died of cancer, and 39 persons who died of other diseases. Robinson et al. (1965) detected no differences in total DDT-derived material or dieldrin between 50 biopsy and 50 necropsy samples. Nor was there any correlation for the necropsy samples between storage levels and cause of death, classified as neoplasm, cardiovascular disease, infection, or accident.

The metabolism of parathion in man, as measured by excretion levels of p-nitrophenol, was influenced by intercurrent disease, and skin and kidney pathology (Davies et al., 1965).

5. *Nutrition.*—In the case of the organochlorine materials, which have been of most interest in this regard, the mass of body fat present in the well-fed animal serves as a protective mechanism by storing the insecticide and, thus, shielding the senstitive nervous tissue from the poison. In the starved animal this protective mechanism is either absent or is present in reduced amount. The lowered liver microsomal enzyme activity of starved animals may influence their susceptibility to poisoning. Furthermore, if the starved animal had already stored a significant amount of one of the organochlorine pesticides prior to starvation, then the amount of insecticide mobilized along with the fat during starvation might be decisive in determining the outcome of a new exposure to the same or a related poison (Dale et al., 1962).

It has been shown that both mammals (Spicer et al., 1947) and fish (Hoffmann and Surber, 1949) which are in good condition, especially those which are fat, are more resistant to DDT poisoning. DDT poisoning in birds is accentuated by starvation. Rats which had been given relatively large dosages of DDT to produce significant fat storage levels of the compound developed characteristic DDT tremors during starvation (Fitzhugh and Nelson, 1947). However, attempts to produce symptoms of poisoning in animals previously dosed with dieldrin by starvation have not been successful (Hayes, unpublished data).

Both the mouse and the rat showed increased toxic effects from DDT when the percentage of fat in the basal diet was increased from 5 to 15 percent (Sauberlich and Baumann, 1947). A reduction in the level of dietary fat to 0.5 percent decreased the toxicity of DDT to both species. All lipids tested, including a highly saturated fat (hydrogenated coconut oil), moderately saturated fats (butter and lard), and highly unsaturated fats (peanut oil or corn oil), produced essentially equal effects. The effect of the fat was not modified by the addition of cholesterol at a dietary level of 0.5 percent. The authors reasoned that the increased toxic effect of DDT when the compound was given in a high fat diet was due primarily to promotion of absorption of the toxicant by the fat. Varying the protein content of the diet produced opposite effects from changes in the fat moiety. Thus, an increased protein content (above 20 percent) tended to be associated with a smaller DDT effect, while a decreased protein content (10 percent) apparently reduced resistance to DDT.

The changes in storage and excretion of DDT in rats as a result of starvation have been studied by Dale et al. (1962). In these starvation tests, mobilization of body fat resulted in an increased concentration of DDT-derived material in that tissue, and a corresponding increase in other tissues studied (plasma, brain, liver, and kidney).

An augmented excretion of metabolites ocurred during starvation in spite of decreased intake of DDT. The increased excretion was inadequate to prevent the increase in concentration of DDT-derived material in the body tissues studied, although it tended to do so. In this work no effort was made to assess the role of the liver microsomal enzymes, or the effect of starvation on their function. However, such a study would be complicated by the apparent large increase in DDT dosage produced by release of the compound from mobilized fat.

The effect of the level of dietary protein on the sub-acute toxicity of dieldrin added to the diet has been studied in rats (Lee et al., 1964). A low (10 percent) protein diet accentuated the toxic effect of dieldrin as measured by increased mortality, increase in liver lipids, decrease in total liver content of vitamin A, and more marked histopathology involving cellular edema and fatty infiltration. However, total liver weight in dieldrin-fed rats was unaffected by a low protein diet but was increased by a high protein (25 percent) diet.

A dietary deficiency of riboflavin or nicotinic acid can accentuate dieldrin toxicity in rats (Tinsley, 1966). Also, dieldrin appears to interact in the metabolism of unsaturated fatty acids and accentuates an essential fatty acid stress.

Dietary DDT at levels of 10 to 100 p.p.m. decreased the utilization of vitamin A and carotene in the rat as measured by liver storage of vitamin A (Phillips, 1963).

6. *Temperature.*—Baetjer and Smith (1956) have reported that parathion is more toxic to mice at higher environmental temperatures. The temperature effect was less marked when the parathion was injected intravenously than when it was given intraperitioneally. The authors interpreted this difference as an indication that variation in the rate of absorption was probably the most important factor causing the increased toxicity at higher temperature. Sarin was more toxic to monkeys at an environmental temperature of 38° C. than at 25° C. following either percutaneous or respiratory exposure (Craig et al., 1959). However, sarin was more toxic to hibernating than to warm, nonhibernating hamsters (Scaife and Campbell, 1958). Rats held at ordinary room temperature withstood 4,000 p.p.m. malathion without showing signs of intoxication (Marton et al., 1962). However, when these rats were clipped and exposed to an ambient temperature of 1.5° C. they survived for a much shorter period in the cold environment than did control animals not receiving malathion.

DDT, warfarin (Keplinger et al., 1959), and azinphosmethyl (Wassermann et al., 1963) are more toxic to rats at 36° C. than at lower temperatures (4° to 8° C.). Warfarin differs from the other compounds tested in that it is least toxic at an intermediate temperature (26° C.)

and then becomes more lethal as the animals are cooled or warmed (Keplinger et al., 1959).

Temperature has also been shown to interact with pesticide exposure in affecting the physical performance of rats. Thus, rats repeatedly fed amounts of DDT in their diet which did not otherwise produce symptoms showed a decreased ability to swim in cold (20° C.) water (Toxicology Section, unpublished data).

It is well known that the nitrophenols, including those used as pesticides, are more toxic at higher environmental temperatures (Sollmann, 1948). The toxicities of DNOC and dinitro-o-phenol to rats are significantly greater at 36° C. than at 10° C. (Keplinger et al., 1959). The same is true of pentachlorophenol. These compounds act by increasing the oxidative metabolism and, therefore, the heat production of the body, chiefly by direct peripheral action (Hayes, 1963).

Some observations in man also indicate an effect of temperature on the toxic effect of pesticides. Volunteers given dermal doses of parathion showed an increase in their excretion of p-nitrophenol associated with an increase in ambient temperature (Funckes et al., 1963; Durham et al., manuscript in preparation).

In studies of workmen exposed to parathion while spraying under field conditions, excretion rates of p-nitrophenol for men with apparently similar contact with the poison were generally higher during the hot days of July than during the cool days of May (Durham et al., manuscript in preparation).

It has long been the observation of physicians and others in certain agricultural areas that poisoning from the organophosphorus insecticides occurs more often in unusually hot weather than under cooler conditions. However, more spraying is done in the warmer weather; therefore, any valid conclusion based on epidemiological data relating to the effect of temperature on poisoning would have to take into account the intensity of exposure and the size of the population at risk.

7. *Light.*—Insofar as pesticides are concerned, light seems to be a much less important environmental factor than is temperature in effect on toxicity.

However, ultraviolet light can catalyze the oxidation of a number of organophosphorus pesticides, including parathion which was converted to the more toxic oxygen analogue, paraoxon (Cook, 1955; Frawley et al., 1958).

Photosensitivity has been demonstrated in rats with hexachlorobenzene-induced porphyria (Pearson and Malkinson, 1965).

Pathology.—With the exception of the neurotoxic effect which occurs with certain compounds, the pathology associated with exposure to the organic phosphorus pesticides is not noteworthy. However, the chlorinated hydrocarbon compounds do produce significant histopathology, particularly of the liver, in animals which are subjected to high levels over long periods of time.

The histopathology associated with pesticides which has caused the most discussion occurs in animals given repeated doses of DDT or of certain other of the chlorinated hydrocarbon compounds. Histological changes occur in the livers of rats even at very low levels of DDT in their diet. These changes were first reported by Kunze et al. (1949). These workers reported that histopathology could be detected in the livers of rats maintained for six months on a diet containing 5 p.p.m. of DDT. However, Cameron and Cheng (1951) were unable to demonstrate any pathology in rats sacrificed after being dosed for more than a year at levels corresponding to food concentrations up to approximately 350 p.p.m. Some other workers, including Deichmann and colleagues (1950) and Treon and Cleveland (1955), reported liver changes induced by relatively low dosages of DDT, while Haag and associates (1948) and Greenwood and coworkers (1953) failed to find such changes. Ortega et al. (1956) reported that liver cell necrosis occurred with dosages in excess of 1,000 p.p.m. but not at lower levels. Histological changes restricted to the liver occurred at levels as low as 5 p.p.m., but liver function, as measured by bromsulphthalein excretion, was not affected in rats fed 400 p.p.m. or less. The histological changes in the parenchymal cells of the liver consisted of an increased deposition of fat, margination of cytoplasmic granules, hypertrophy of the cells, and most characteristic—the formation of complex, lipoid cytoplasmic inclusion bodies termed "lipospheres". Ortega (1962) has more recently reported additional details of these cytoplasmic alterations as noted in studies using both light and electron microscopy.

The similarity in both etiology and appearance of these histologic changes with those characteristic of microsomal enzyme induction have led some observers (Ortega, 1962; Ferrigan et al. 1965) to label them as adaptive rather than as a pathologic process. The DDT-induced histologic changes have never been correlated with hepatic dysfunction. The changes in liver cell morphology occur in the rat at a much lower dietary level of DDT than do other "toxic" effects. The fact that histological changes similar to those noted with DDT occur with phenobarbital and are correlated with enzyme induction suggests a similar view for the DDT-produced changes. There are several bits of experimental evidence to support such a view. Ortega (1962) has noted that chronic feeding of DDT produced a considerable increase in the amount of SER in the liver cells with a partial displacement of rough-surfaced reticulum. Most significantly, Ferrigan et al. (1965) have pointed out that, for rats fed dieldrin in their diet, these specific structural changes in the liver were related more to intensity and duration of exposure than to intoxication. In fact, the liver changes were not found in intoxicated rats, only in unintoxicated ones.

Monkeys develop liver histopathology only with relatively high dosage levels of DDT (Durham et al. 1963). No liver histopathology occurred in monkeys fed DDT at dietary levels of 200 p.p.m. or less for periods of up to 7.5 years. One of six monkeys fed 5,000 p.p.m. of DDT did develop the cytoplasmic inclusions which have been characteristically associated with chlorinated hydrocarbon poisoning in the rat.

Chlorinated hydrocarbons other than DDT also produce these liver changes in rats (Ortega et al. 1957). The lesions were produced at minimum dietary levels of 2.5 p.p.m. for dieldrin and chlordane and 50 p.p.m. for lindane and toxaphene.

It is generally agreed from studies in a wide variety of species of animals that large doses of DDT can cause liver cell necrosis (Hayes 1959b), but this effect must not be confused with the reversible effects of small doses discussed above.

It is interesting to note that, in rats poisoned with large doses of chlorinated hydrocarbon pesticides, increases in liver weight and in liver fat content have been noted for DDT (Sarett and Jandorff 1947) and for dieldrin (Durham et al., manuscript in preparation).

DDT has been shown to cause atrophy of the adrenal cortex (Nelson and Woodard 1949). This finding is of particular interest in view of the fact that, in animals exposed to DDT, high levels of DDT are stored in the adrenal gland in comparison with other tissues. This effect has been shown to be due to the o,p′-DDT isomer present in the technical product (Cueto, Brown, 1958).

Storage.—Pharmacodynamics is being considered in a separate section of this report. However, the good correlation between tissue storage for the chlorinated hydrocarbon pesticides between experimental animals and man is worthy of brief comment here.

On a constant dosage of DDT both experimental animals (Hayes 1959) and man (Hayes et al. 1956) show increased fat storage of the compound for a time but eventually reach a plateau level beyond which further accumulation does not take place even though dosage is continued. Loss of the compound from storage is slow in both experimental animals and man. The rate of loss is proportional to the storage.

Induction of liver microsomal enzymes.—One of the important pharmacologic properties of the chlorinated hydrocarbon pesticides is their capacity to stimulate activity of the drug-metabolizing enzymes in the liver microsomes. This effect was first noted for chlordane among the chlorinated pesticides (Hart et al., 1963) but since has been reported for an additional number of these compounds.

There also seems to be considerable variation between different phyla, classes, groups, and species of animals with regard to microsomal enzyme activity. In some species, such as the rat, there are sex-related differencess in enzyme level. There are also differences between aquatic and terrestrial animals. For example, these enzymes seem to be almost completely lacking in fish (Brodie and Maickel 1962). Many of the differences between various species with regard to susceptibility to certain chemicals including pesticides, can be explained on the basis of these differences in microsomal enzyme activity.

The microsomal enzymes are essentially absent in the newborn, but build up rapidly in the early days or weeks of life (Fouts and Adamson 1959).

The changes in activity of the liver microsomal enzymes have been shown to be accompanied by characteristic changes in the ultrastructure of the liver cell. Thus, Fouts (1962) and Remmer and Merker (1963) have shown that increases or decreases in liver microsomal enzyme activity are paralleled by similar changes in the amount of SER in the liver cells. Fouts and Rogers (1965) have published excellent photomicrographs which illustrate the differences between normal liver cells and liver cells from enzyme-stimulated (phenobarbital-treated) rats. As discussed above under pathology, these histologic changes are thought by some observers to be identical with those caused by low levels of DDT and other chlorinated hydrocarbon pesticides and labelled as pathologic.

Although the matter of interaction is being considered by another reviewer, one aspect of this subject shows so beautifully the extension of animal findings into man that it deserves to be mentioned here.

Street (1964) reported that the storage of dieldrin in fat of female rats was markedly depressed when DDT and dieldrin were fed simultaneously. On the other hand, addition of methoxychlor to the diet did not affect the dieldrin storage pattern. A similar effect on fat storage of dieldrin in the rat was also produced by certain drugs, including phenobarbital (Cueto and Hayes 1965), aminopyrine, tolbutamide, phenylbutazone, and heptabarbital (Street et al. 1966).

Very recently, Davies et al. (1969) have described lowered blood levels of DDE in patients taking the anticonvulsant drugs phenobarbital and diphenylhydantoin (dilantin). The microsomal inductive effect of DDT has been utilized therapeutically by Thompson et al.

(1969) in the treatment of a case of familial unconjugated nonhemolytic jaundice with DDT.

Reproduction.—There is now a considerable volume of data available on the effect of pesticides on reproduction in experimental animals. A two generation study in two species is required for compounds to be granted a tolerance level in food. In general, the results of these studies have been reassuring. In few instances have effects on reproduction occurred in the absence of other signs of toxic effect.

In addition to any possible effects on reproduction, DDT and the other chlorinated hydrocarbon pesticides may also poison the young by way of the mother's milk, since these compounds are excreted in the fat moiety of the milk in exposed animals.

Some workers have postulated an estrogenic effect for DDT or its congeners based on general steric similarity to diethylstilbestrol. Burlington and Lindeman (1950) showed that DDT produced a striking inhibition of testicular growth and secondary sexual characters of cockerels. Welch et al. (1969) reported that technical DDT; p,p′-DDT; o,p′-DDT; and methoxychlor had estrogenic effects in the rat as shown by an increase in uterine net weight.

However, Fisher et al. (1952) concluded that p,p′-DDT did not have estrogenic activity since it failed to maintain estrus in ovariectomized rats, although an analog (2,2′-bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane) did show such activity.

In addition to this possible direct estrogenic effect, DDT and the other chlorinated hydrocarbon pesticides may affect reproduction through their microsomal inductive activity. This increase in drug-metabolizing enzyme activity is accompanied by an increased conversion of steroids to polar metabolites (Kupfer, 1969; Welch et al., 1969). The significance of this effect for mammalian reproduction has not yet been shown. However, it has been postulated (Wurster 1969) that this action is responsible for the decreases in eggshell thickness observed in certain bird species fed chlorinated hydrocarbon pesticides (Stickel, 1969).

There have been reports of a number of wild bird species, notably the peregrine falcon and the bald eagle, which are showing declining reproductive success and population numbers. This decline has been attributed to chlorinated hydrocarbon pesticides by some observers (Risebrough, 1969; Wurster, 1969). The estrogenic and enzyme effects noted above indicate a possible mechanism for such an etiology. However, there seems at this time to be a very reasonable doubt that residues of the chlorinated hydrocarbon pesticides are found in the natural feed of these birds at levels equivalent to the dosage necessary to produce these effects.

In studies with the developing chicken embyro involving more than 400 chemicals, including a number of pesticides, high and specific teratogenic activity was noted with 3 fungicides—captam, folpet, and difolatan (Verrett et al., 1969). Captan has also produced mutagenic effects in bacteria, in a heteroploid human embyronic lung cell line, and in a cell line derived from the kidney of the rat kangaroo (Legator et al., 1969).

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PREVENTIVE AND THERAPEUTIC MEASURES

Since the end of World War II, many new chemicals for the protection of crops and the abatement of nuisances arising from the presence of various types of pests have come into use. Pesticides, because they are designed to injure some living species, may have a potential for harming man. Such harm may arise as a result of use of consumption of stored or agricultural products treated with pesticidal chemicals or of handling such chemicals during their application to stores or produce. Here, we shall be concerned only with risks to man from the handling and application of pesticidal chemicals.

California, the State that uses about one-fifth the total amount of pesticidal chemicals consumed within the entire United States per year, requires its physicians to report attendance upon any injured worker (section 6407 of the California Labor Code). This system has allowed the Bureau of Occupational Health of the California Department of Public Health to report that, during the years 1965 and 1966, the greatest number of occupational illnesses attributed to economic poisons was held to be due to organophosphorus compounds. The next highest number of illnesses (about 79 percent of that attributed to organophosphorus compounds) was due to herbicides, with phenolic compounds, halogenated hydrocarbons, fungicides, compounds containing Pb or As, organomercurial compounds and carbamates following in order of decreasing activity in causing sickness.

Although the experience of California with pesticidal chemicals may not be typical of the entire United States (in New York State, the majority of poisonings by pesticides reported by Poison Control Centers within the State are due to insecticides; rodenticides cause about 25 percent of the reported cases and herbicides only about 4 percent whereas in California herbicides initiate apparently about 28 percent of the illnesses), that State's figures for the incidence of disease due to economic poisons may serve as a guide to what the other States may expect as their agricultural activities become more intensive in response to the need to provide food for a progressively increasing human population.

In the total of illnesses caused within California by economic poisons, the most common type of effect was the development of some sort of skin condition, with an eye condition, a generalized intoxication, a chemical burn and a respiratory condition following in order of decreasing incidence. When one considers individual types of economic poisons, one finds that herbicides and phenolic compounds have similar hierachies of effects, with an eye condition leading the other sorts of actions as the most common and being followed in order by a skin condition, a chemical burn, a respiratory condition and a generalized intoxication. The other 6 types of economic poisons have various, and possibly characteristic, hierachies of toxic actions. All these heirarchies are summarized below, in units of the incidence of the least common effect for each type of economic poison.

	Skin condition	Eye condition	General Intoxication	Chemical Burn	Respiratory condition
Organophosphorus derivatives	21. 5	13. 5	149. 0	1. 0	3. 5
Halogenated hydrocarbons	4. 8	4. 2	3. 0	1. 4	1. 0
Pb or As derivatives	4. 5	2. 0	3. 0	1. 5	1. 0
Herbicides	12. 4	19. 0	1. 0	6. 4	1. 2
Organo-Hg derivatives	4. 0	2. 0	1. 0	8. 0	--------
Fungicides	10. 5	8. 5	1. 0	2. 5	3. 0
Phenolic compounds	26. 0	32. 0	1. 0	20. 5	2. 0
Carbamates	1. 0	--------	1. 0	--------	--------

This compilation shows that the only type of economic poison that is especially likely to cause generalized intoxication is the organophosphorus derivatives. Either the skin or the eye is the part of the body most likely to be injured by most of the other types of economic poisons, with a chemical burn being the most common type of injury by the organomercurials. Hence protection of the eyes and skin from accidental contact with pesticidal chemicals is important for all principal types; in the case of organophosphorus compounds especially, protection from inhalation or ingestion of pesticides is important also.

1. Preemployment examinations.—Because the skin is at least to some extent a barrier that denies access to the interior of the body by external influences and because the skin is injured also by many economic poisons, a normal, healthy skin without a previous history of exanthematous or other sorts of pathologic changes should be a primary requirement in any preemployment medical examination of a prospective employee who may be exposed more or less continuously to pesticides. Similarly, the eyes should be examined carefully by the use of fluorescein or other appropriate dye for evidence of previous ulceration or damage.

Because both the organophosphorus and the halogenated hydrocarbon compounds can induce the electrical seizure patterns in the EEG, an EEG examination, including voluntary overbreathing, should be a part of any thorough preemployment physical examination of a prospective pesticide operative. Good general physical condition of a new pesticide operative should be assured medically; this involves the examiner's taking special care to assure himself that the respiratory and cardiovascular systems have not been damaged in any way and that hepatic, renal and gastrointestinal functions are normal.

2. Personal protection against exposure.—Although employers can institute rules and procedures for the safe handling of pesticides, the individual worker is the final arbiter of his own safety when using these poisons. In particular, he must understand both the need for, and the correct use of, such protective clothing as rubber gloves, rubber boots, rubber apron, impermeable raincoat, goggles, face shield, impermeable hood, dust mask and rubber face mask with protective cannister.

It is the responsibility of the employer to assure that his personnel understand the correct use, and indications for the use, of the protective items mentioned above and that the articles appropriate for the work that the employee will be required to perform are available to him. It is the responsibility of the employee to conscientiously use the protective items and procedures appropriate to the work that he performs. The pesticide operative's supervisor has responsibility for assuring both that the employee knows the proper use of the protective items made available to him and that the employee makes correct use of the protective items and procedures that pertain to his handling of particular pesticides.

Whenever the more potent organophosphorus compounds (such as demeton, or systox, and dimefox) are handled or any potent pesticide is applied as an aerosol within a greenhouse, a full face mask with a protective cannister and an impermeable hood should be worn. In other situations, goggles, a face shield or a dust mask, as appropriate, is usually sufficient protection to the head and neck region.

For protection of the body in general from the most toxic materials, boots, rubber gloves and either an impermeable coat or a tightly-woven coverall and a rubber apron should be worn whenever concentrated pesticidal chemical must be handled by the employee. After the concentrated material has been diluted for use, spraying from the ground should be performed when wearing rubber gloves, rubber boots, and an impermeable coat or, at the least, a coverall and rubber apron. If spraying is done overhead, an impermeable hood should be worn in addition to any other protection of the head and neck region.

The pesticide operative should be acquainted with several additional general procedures relating to his own safety during mixing and spraying operations:

1. Do not smoke during diluting and spraying operations. Cigarettes or other items of tobacco should not be carried on the person during these operations.
2. Wash face and hands well before smoking, eating or drinking during the workday.
3. Shower or bathe thoroughly at the end of the workday.
4. Do not try to clear a blocked nozzle by sucking or blowing with the mouth.
5. Avoid spray drift.
6. Clean protective clothing and equipment frequently and store carefully to avoid tearing or creasing that may lead to breaks. Permeable clothing should be laundered after each day's use, at least. In case of a spill onto such clothing, the contaminated article should be removed at once and replaced by a fresh item.

A number of studies have been made of exposures of industrial and agricultural workers to pesticides (*1–16*, for example). These find in general that industrial workers suffer more intense exposures to pesticides than agricultural workers. In a study of workers using DDT under various situations, Wolfe, et al. (*17*) found that a man spraying DDT inside a house received a total exposure to this chemical about 7.3 times that of a man applying the same spray outside a house, receiving about 64.5 times as great an exposure by the respiratory route and about 7.2 times as much by dermal contamination. Durham and Wolfe (*3*) reported that a man spraying apple trees with DDT had essentially the same exposure to that insecticide as a man spraying outside a house for vector control. Dermal contamination contributed 2,246 times as much exposure to DDT in these outside situations as inhalation. Durham and Wolfe (*4*), in a similar study with parathion, found that dermal contamination contributed about 1,550 times as much exposure to this organophosphorus compound as inhalation. When aerosolized chlorthion was dispersed, Culver, et al. (*19*) found that cutaneous contamination was only 10 times as important as inhalation as a route of exposure. When a mist of parathion was sprayed on tomato plants, Simpson and Beck (*19*) found that cutaneous exposure was slightly less than 31.4 times as contributory to intoxication as was inhalatory exposure.

Intoxication by pesticides is a particular hazard to aeroplane pilots engaged in custom application of such compounds because each man's safety depends on his being constantly alert and vigilant in making the small adjustments required to keep the plane flying at the proper altitude in its flight pattern. In an analysis of accidents to aerial appliers of agricultural chemicals between 1963 and 1966, Reigh and Berner (*10*) reported that the accident rate for aerial appliers was three times as high as that for such commercial flying as operation of an air taxi. Factors relating to the condition of the pilot were considered causative in 53 to 70 percent of all crashes during the 4 years of the study. In 12 fatal crashes studied, the blood cholinesterase levels of the pilots were significantly lowered in 8. The importance of this factor is emphasized by the cases reported by Smith (*11*), which illustrate the value of the pesticide operative's being aware of the significance of subjective changes. As an example, one case reported by Smith was of a pilot who had experienced blurred vision and extreme fatigue for 3 weeks but refused to take time off or to have the cholinesterase activity of his blood checked. The pilot lost control of his plane at about 200 feet and crashed; he died 3 days later, but of pesticide poisoning rather than of trauma.

The experiences summarized in the two preceding paragraphs reinforce the general suggestions stated previously for safe handling of pesticides. They mirror particularly clearly the importance of protection of the skin from contamination by pesticides in liquid preparations and of protection of the respiratory system whenever pesticide aerosols are used, especially when such aerosols are generated within enclosed spaces.

3. Personal indicators of overexposure.—At the present time, the two groups of chemicals used most widely as pesticides are the chlorinated hydrocarbons and the inhibitors of cholinesterase (including organophosphorus, carbamate and bisquaternary amine types). Types of pesticides of some importance are thiocyanates, dinitro derivatives of phenol and cresol, anticoagulant compounds, fluoracetate and fluoracetamide, such herbicides as di (*p,p'*-N-methylanilinium chloride), or paraquat, and a number of others. The characteristics of poisoning by pesticides of various types are summarized in handbooks (*20–22*, for example). No attempt will be made to summarize them here, but emphasis is placed on the importance of the worker's taking steps to familiarize himself with the effects to be expected from the materials with which he works. The worker must be impressed with the ideas that a stout heart and strong muscles to do not necessarily protect against poisoning by pesticidal chemicals, that prudence in taking proper measures to prevent poisoning is good sense rather than cowardice, and that recognition of incipient poisoning in himself and seeking appropriate medical assistance, or avoiding further contact with the offending chemical for a time, is the course of astuteness and wisdom rather than of courage.

For the two most widely used groups of pesticides, the most usual early symptoms and signs are:

Chlorinated hydrocarbons: Hyperexcitability, nausea and vomiting, tremors, depression, dermatitis, urticaria, and the results of liver and kidney damage.

Inhibitors of cholinesterases: Running nose, sensation of tightness in the chest or of shortness of breath, cough, dimmed or blurred vision, tearing, headache, drowsiness, dreaming and disturbed sleep, increased fatiguability and inability to concentrate attention on a given task.

4. Clinical diagnostic techniques.—The diagnosis of poisoning by pesticides requires no particularly unusual techniques other than the use of specific analytic methods to detect individual compounds. The general problems of diagnosis of poisoning by pesticides have been discussed (*23*, *24*).

The chlorinated hydrocarbon pesticides are stored in the body fat of people in various countries of the world to varying degrees, but residues of these materials appear even in Alaskan Eskimos (*25–34*, for example). A method for preparing samples of tissue and fat for examination for pesticides has been published (*35*). The methods of thin-layer chromatography of Kovacs (*36*) are extremely sensitive and are useful in detecting small amounts of chlorinated hydrocarbon and organophosphorus compounds, respectively, in biological samples.

Although the chlorinated hydrocarbons are stored in fat, which can frequently be sampled readily by simple aspiration, their concentration there bears no fixed relation to the degree of intoxication exhibited by the individual (*37*). The severity of intoxication seems to be correlated, at least in the rat, with the concentration of chlorinated hydrocarbons in the brain (*38*). Blood and urine are biological fluids that are more closely in chemical equilibrium with the brain than is body fat. Therefore, methods for estimating the concentrations of these materials in urine and blood have been sought. Durham et al. (*39*) found that the urinary excretion of DDA, a metabolic product of DDT, by 39 subjects from the general population was about 4 percent of that by 40 workers in formulating plants and about 32 percent of that by eight men who ingested 3.5 mg./day of DDT. Durham et al. concluded that a more sensitive method was needed, because their method gave values below the limit of sensitivity for about three-fourths of subjects from the general population. Cueto and Biros (*40*) described a more sensitive gas chromatographic method, with which they were able to identify 11 chlorinated hydrocarbon insecticides. This method indicated that people in the general population excrete in their urines about 70 percent as much total DDT congeners as a group of people exposed to DDT occupationally. The general population excreted in its urine slightly more than 3 percent as much dieldrin as a group of occupationally exposed men.

The urine has been found to be a reasonably good indicator of exposure to a number of other types of pesticides: arsenic (*41*), lead (*42*), mercury (*43*), DNOC (*44*), DNOSBP (*14*), EPN (*45*), PCNB (*46*) 2,4-D (*47*), 2,4,5-T (*48*), atrazine (*48*), Baygon (*49*), carbaryl (*2*), Ciodrin (*50*), p-dichlorobenzene (*51*), Kuron (*48*), malathion (*52*), methyl parathion (*45*), parathion (*45*), Silvex (*48*), Simazine (*46*), and Zectran (*53*). All these methods may not have been applied to human urines, but they should be so applicable with some modification, perhaps.

In some cases, analyses of urine may afford a more sensitive index of the presence within the body of significant concentrations of toxic chemicals than analyses of blood even. This seems to be true particularly of parathion (*54*) and to a lesser extent of the other organophosphorus compounds that yield p-nitrophenol on hydrolysis (EPN and methyl parathion). The urinary excretion of p-nitrophenol can be a valuable indicator during the treatment of poisoning by parathion, or EPN or methyl parathion, of the continued need for therapy until the excretion of this metabolite falls to a low level. In the case of malathion, urinary excretion of ether-soluble organic phosphate is proportional to dosage (*52*) and also affords a useful monitoring method for deciding whether it is safe to discontinue therapy of serious poisoning at a particular moment.

Saliva is another readily obtainable biological fluid that is to some extent equilibrated with the blood. Heavy metals, including mercury, appear in the saliva (*55–57*). Joselow et al. (*6*) found a high correlation between the concentrations of mercury in parotid saliva and in blood, suggesting that saliva may be of diagnostic value in detecting and treating poisoning by organic mercurial fungicides. These compounds have caused severe and fairly common poisoning in Sweden, where they seem to have been used to a greater extent than in this country. Early signs of poisoning by mercury, which should be known to anyone who uses organic mercurial fungicides on repetitive occasions, include fine tremors of the hands, loss of peripheral vision, incoordination of speech, gait and stereognosis, and headache and irritability.

In some cases, analysis of blood for pesticidal chemicals may be an important aid to recognition of the cause for an intoxication. Probably the greatest emphasis has been placed on the development of analytical methods for chlorinated hydrocarbon insecticides, but methods are available also for such compounds as pentachlorophenol (*58*), DNOC (*44*) and 2,4-D (*59*). Dale et al. (*60*) described a method whereby eight different chlorinated hydrocarbon chemicals could be detected in blood samples taken from representatives of the general population or from subjects occupationally exposed to aldrin and dieldrin. A more efficient method for determination of chlorinated insecticides in blood was described by Dale et al. (*61*). Another modification of the method of Dale et al. (*60*) was used by Edmundson et al. (*62*), who found that the concentrations of DDT and DDE in blood samples from nonwhite subjects were consistently higher than those from white subjects in the same occupational groups. Curley and Kimbrough (*63*) reported that human milk may contain about 18 times the concentration of DDT in plasma, something more than seven times the DDE, about 50 times the DDD, about 13 times the total DDT congeners, about six times the BHC, only slightly more heptachlor epoxide and about eight times the dieldrin.

In poisoning by inhibitors of cholinesterases of any chemical type, measurement of the cholinesterase activities of plasma and red cells may be a useful procedure. Differential inhibition of cholinesterases of the pseudo and true types can be helpful in recognizing the cholinesterase inhibitor involved in addition to giving some rough indication of the severity of poisoning. When organophosphorus compounds are involved, any of a number of methods for estimation of cholinesterase activity may be used; when a reversible inhibitor is concerned, the cholinesterase activity is estimated the most accurately by the pH-stat method (*64,65*).

Symptomatology is another important aspect of diagnosis of intoxication by economic poisons. In a study of illnesses reported from 36 States by 1,105 employees of pest control companies, Stein and Hayes reported (*12*) that the most common complaints were dermatitis, infection (including pneumonia), headache, gastrointestinal upset, cardiovascular disturbances, joint pain, and dizziness. In a somewhat similar study, Reich, *et al.* found (*66*), that the most common signs and symptoms of 129 cases of poisoning by pesticides in southern Texas were vomiting, nausea, miosis, weakness, abdominal pain, dizziness, sweating, increased salivation, headache, tachycardia, and hypertension. Most of the second set of signs and symptoms of poisoning by insecticides are referable to inhibitors of cholinesterases whereas the first set contains symptoms that could arise from intoxication by chlorinated hydrocarbon insecticides as reasonably as from that by inhibitors of cholinesterases.

The greatest uncertainty in the diagnosis of poisoning by pesticides relates to hypersensitivity. As reported by Stein and Hayes (*12*), dermatitis was the most common complaint by pesticide appliers working in 36 different States. West reported (*13*) that dermatitis was among the most frequently reported diseases of farm laborers. The differentiation of dermatitis arising from hypersensitivity to a chemical from that depending upon a direct irritant or other cutaneous effect of a chemical taxes the diagnostic ability of the dermatologist, yet the distinction is important because the methods of treatment differ widely in these two situations.

The demonstration by Bleiberg *et al.* (*67*) of both chloracne and photosensitive hepatic porphyria in workers who had been exposed to 2,4-D and 2,4,5-T is taken to show the involvement in the dermal effects of these two pesticides of both direct effects on the skin and of photosensitization of the skin. The latter effect results from deposition of uroporphyrins in the skin as a result of damage to the liver. The same sort of effect has been reported from Turkey when flour made from wheat grown from seed treated with BHC was ingested. This toxic form of photosensitive hepatic porphyria can be differentiated from the hereditary form of the disease by estimation of the concentration of delta-aminolevulinic acid in the urine. This will be normal in the presence of markedly increased concentrations of uroporphyrins and coproporphyrins in the toxic form of the disease but markedly elevated also in the hereditary form.

5. Therapy.—Treatment for intoxications by most classes of pesticides is nonspecific and oriented around the signs and symptoms observed in the patients under treatment. Good diagnosis of the cause of the signs and symptoms is important, however, because, to give one definite example, use of atropine to treat poisonings by the pesticides dinitrophenol and pentachlorophenol, which resemble those from inhibitors of cholinesterases in having as symptoms sweating, marked fatigue, nausea, vomiting, occasional diarrhea, and convulsions, may be rapidly fatal whereas it can be lifesaving in actual cases of poisoning by cholinesterase inhibitors. Probably the best indicator for differentiating poisoning by the nitro or chlorophenols from that by inhibitors of cholinesterases is that a definite to severe hyperpyrexia will occur in poisoning by the phenol derivatives whereas the body temperature in poisonings by the inhibitors of cholinesterases tends to be depressed, at least in the acute type of poisoning.

In any poisoning in which the causative agent may have entered the body through the mouth, gastric lavage with several liters of water is indicated. In a conscious subject, the same result may be accomplished by administration of syrup of ipecac or some other emetic. Catharsis by sodium sulfate (30 gm. in 250 ml. of water), may be useful also. A slurry of activated charcoal (5–6 heaping teaspoons of activated charcoal in 200 to 250 ml. of water) may be placed in the stomach after cessation of vomiting or gastric lavage in the absence of any more definitive treatment.

When the causative agent of poisoning may have contaminated the skin, as during spraying operations, the skin should be well scrubbed with soap and water. The administration of oxygen or artificial ventilation are obvious therapeutic procedures in cases of anoxemia and cyanosis or apnea, respectively.

In treating poisonings by the chlorinated hydrocarbon insecticides, sympathomimetic compounds, such as epinephrine, are contraindicated because of the danger that they will induce ventricular fibrillation. Useful drugs are such central depressants as phenobarbital and pentobarbital. Diphenylhydantoin also may be of value in controlling convulsions. Calcium gluconate may be useful. Patients who convulse should be observed carefully for several days to a week for signs of secondary or persistent effect. EEG recordings are useful in detecting prolonged cortical dysfunction but are not useful *per se* in diagnosis of poisoning; inhibitors of cholinesterases and chlorinated solvents (carbon tetrachloride, tetrachlorethylene, etc.) may yield EEG patterns similar to those due to the chlorinated hydrocarbon insecticides. Repeated administrations of barbiturates and anticonvulsant drugs may actually reduce the body burden of chlorinated hydrocarbon insecticides (*68*).

Materials that may be useful in treating poisonings by other pesticides that have no definitive therapies include amyl nitrite (pearls), cold water (for rectal and colonic irrigation in hyperthermia), glucose (sterile 5 percent solution), morphine, phentolamine, sodium nitrate (sterile 3 percent solution), sodium sulfate (sterile 10 percent solution as well as crystals or powder), and sodium thiosulfate (sterile 10 percent and 25 percent solutions).

Only three classes of pesticides can be said to have fairly specific therapy for poisonings by them: The inhibitors of cholinesterase, the anticoagulant rodenticides, and heavy metal compounds. The anticoagulant rodenticides have as an important part of their toxic actions inhibition of the production of prothrombin by the liver, although this may not be their sole mechanism of action (especially true for the indanedione derivatives). Vitamin K_1 (phytonadione), is a specific antidote for the hemorrhagic effects of these compounds. This may be administered by slow intravenous drip. Initially, in severe poisoning, transfusion may be desirable to furnish an increased concentration of prothrombin extrinsically until the liver can be enabled by the administered phytonadione to increase the supply of intrinsic prothrombin. Ascorbic acid and ferrous sulfate may be useful adjuncts to phytonadione in recovery from serious poisoning by an anticoagulant rodenticide, particularly so when the poisoning has entailed significant loss of blood.

Poisonings by heavy metal derivatives (of arsenic, copper, lead, manganese, mercury, and zinc), can be combatted in two ways: By preferential affinity and removal from active sites in tissues, and by chelation, with the formation of complexes from which the metallic atom is no longer free to diffuse. Dimercaprol (BAL) accomplishes both these mechanisms of antagonism for such metals as arsenic and mercury that seem to react with sulfhydryl groups in target tissues or organs. Calcium disodium edetate (EDTA) and penicillamine are less specific than dimercaprol and accomplish only the second of these general mechanisms of antagonism to heavy metal poisonings. Dimercaprol is particularly useful in poisonings by preparations containing arsenic or mercury; its effectiveness in lead poisoning is questionable. Dimercaprol is injected intramuscularly as a solution in oil. EDTA is administered preferably by intravenous or intramuscular injection in serious poisonings, but can be given by mouth. With the last route of administration, chelation within the intestinal lumen of lead, for example, may result in enhanced absorption of the metal. Copper, lead, manganese, mercury, and zinc can be effectively detoxified by chelation by EDTA. Penicillamine is a less versatile chelating agent; after oral administration, it is effective in removing copper and lead, and possibly mercury, from the body. Even more specific is deferoxamine, which seems to be capable of chelating only iron. It can be given by either intramuscular injection or oral administration to treat acute intoxication by iron, which occurs in the fungicide Ferbam but is not a usual component of pesticidal chemicals.

The final group of compounds with more or less specific therapeutic measures available is that of inhibitors of cholinesterases. This group is heterogeneous chemically although all its members have a common mechanism of action. It includes bisquaternary amines, carbamates and organophosphorus derivatives. The first two subgroups differ from the third one in that inhibition of cholinesterases by them is more readily reversible than that by the organophosphorus compounds. In all cases, a prominent, although perhaps not the only, source of toxic activity is accumulation of acetylcholine within the blood and various tissues of the poisoned individual. Atropine is a competitive antagonist of acetylcholine at many nerve endings in effectors of the body (especially in muscarinic effectors innervated by the parasympathetic division of the autonomic nervous system and less so within the central nervous system). Atropine is an effective antagonist of poisoning by many inhibitors of cholinesterases up to the level of intoxication by 2 to 3 LD_{50} doses, where effects on nicotinic effectors (particularly neuromuscular junctions) by the accumulated acetylcholine become important. When this generalization is untrue, as in the case of carbaryl, one has to think that the pesticide has some other important mechanism of action. Atropine will antagonize only about 1.4 LD_{50} doses of carbaryl.

To antagonize the paralysis of muscles caused by doses of inhibitors of cholinesterase above about 2.5 to 3 LD_{50} doses, the oximes have come into use. The only one of these compounds available for clinical use within the United States is N-methyl, 2-formyl pyridinium oxime, employed as the chloride (2–PAM Cl), the bromide (2–PAM Br) or the methane sulfonate (P2S) or "Contrathion". Also available and used fairly widely in Europe and the Far East is Toxogonin (bis [N-methyl, 4-formyl pyridinium oxime] ether), used as the dichloride or dibromide salt. In intoxications by some inhibitors of cholinesterases, Toxogonin seems to be effective even when 2–PAM is only partially effective (*69*). The mechanisms of action of both 2–PAM and Toxogonin in overcoming block of neuromuscular transmission seem to be that the oximes reactivate the inhibited cholinesterase by being themselves phosphorylated by the phosphoryl group that previously had inactivated cholinesterase by reacting with its active site. The phosphorylated oximes in some cases are themselves potent inhibitors of cholinesterases, so that stable phosphorylated oximes may induce a secondary poisoning having the same characteristics as that induced originally by the organophosphorus insecticide. In many cases, however, the phosphorylated oximes are unstable and undergo hydrolysis within the body, the products of this hydrolysis then being excreted in the urine. In this way, the oximes in many cases accelerate the loss from the body of the phosphoryl moiety that inactivates cholinesterase in various tissues and organs.

The statement has been made that the oximes should not be used in treating intoxications by carbamates. This is nearly as untrue as the generalization that oximes should be used in treating intoxications by all organophosphorus anticholinesterase compounds. In poisonings by some carbamates, the oximes seem to be quite useful; in poisonings by some organophosphorus compounds, the oximes are almost completely ineffective and in a few cases (morphothion, for example) seem to enhance the toxicity of the organophosphorus compound. What is needed, therefore, rather than a blanket approval of the use of oximes in treating intoxications by organophosphorus compounds and a blanket proscription against their use in intoxications by carbamates, is precise knowledge of the intoxications in which the oximes may be expected to be useful, of those in which the oximes accomplish neither harm nor benefit and of those in which the oximes are likely to be dangerous.

Because of the phenomenon of aging of phosphorylated cholinesterase, whereby the phosphoryl moiety loses one of its alkoxy groups in exchange for a hydroxyl group and becomes inaccessible to such a nucleophilic molecule as an oxime, the oximes become progressively less effective antagonists of the toxic actions of some of the organophosphorus insecticides and miticides. For optimal effectiveness in treating appropriate poisonings wherein marked weakness is evident, oxime must be administered as early as possible after poisoning. The administration of oxime should always be coupled with, or at least followed closely by, the use of adequate doses of atropine. In marked or severe intoxication by inhibitors of cholinesterase, the doses of atropine that are adequate may be very large by usual standards (20 to 50 or more mg of atropine sulfate to a patient during the first day or so of treatment). The objective in using atropine is to produce and maintain a comparatively dry mouth and a dry skin.

In experiments with mice, Andrews and Miskus (7D) have reported that tetraethylammonium chloride is less damaging than atropine when used to treat poisoning by single oral LD_{50} doses of the carbamates Zectran, Lannate and NIA–10242. The toxic effects of another carbamate, Matacil, that produced delayed deaths (12 to 20 hours after oral administration of the carbamate) were not antagonized by tetraethylammonium chloride. The latter material was ineffective against the organophosphorus compound parathion also. Tetraethylammonium chloride seems not to have been used in treating intoxications by anticholinesterase compounds in man.

(*For a more detailed discussion of the therapy of intoxication by inhibitors of cholinesterases, see reference 71.*)

6. Surveillance and epidemiologic studies.—Although acute poisoning by pesticides has received considerable attention and there have been a few studies of occupational groups that were exposed to pesticides during long periods as a consequence of their chosen employment, no systematic study of this sort on a countrywide basis has yet been undertaken. The objectives of such a study might be:

1. To characterize localities throughout the counrty on the basis of their experience with sickness due to exposure to pesticides.
2. To identify the factors related to usage of pesticides that determine differences between localities.
3. To identify the factors related to personnel that determine differences between localities.
4. To identify the factors related to each environment that determine differences between localities.
5. Within individuals localities, to identify the factors relating to usage of pesticides that determine differences between persons.
6. Within individual localities, to identify the factors relating to

separate persons that determine the different experiences of these persons with pesticides.

7. Within individual localities, to identify the factors relating to the environment of the person that determine differences between persons in their experience of sickness due to pesticides.

Two situations seem necessary for collection of the data required for such a study:

1. That poisoning be made reportable in the same way that infectious disease is reportable.
2. That each locality be provided with at least one team of two observers, one of whom would be trained in the economic aspects of the usage of pesticides and who could judge whether the right pesticide was being used at the proper period, the correct frequency, and the appropriate dosage and method of application to achieve control of the target pest. The other member of the team would be trained in the health aspects of the usage of pesticides and would be able to judge whether proper precautions in the handling of pesticides were taken, whether people were affected by pesticides despite their protestations to the contrary or their lack of direct contact with such chemicals and whether effects of pesticides on individuals resulted from direct toxicity, from allergy or from anaphylaxis.

In the reporting of sickness due to pesticides, not only should the fact of illness be reported but also the exact locality and circumstances of the inception of the illness. Necropsies, whether or not death was attributed to pesticides, should include examinations of blood, urine and selected tissues for pesticidal chemicals. At the same time, attempts should be made to characterize the immediate environment of the deceased for its content of pesticides. To quote Dr. Simmons (*72*), "We need to know ——— who gets poisoned with what, and where, when, how and why."

Selby *et al.* (*73*) have attempted to use a measure derived from answers to a questionnaire as an index of exposure to pesticides but found that there was no association between an individual's calculated exposure and analytical values for pesticide in blood, adipose tissue or placental tissue. They conclude that the impact of pesticides upon people in a general population must be assessed on a basis of analytical values rather than of memories and impressions of exposure to pesticidal chemicals. This is useful guidance in the design of a surveillance program in support of an epidemiologic study.

7. Conclusions.—The foregoing discussion has attempted to summarize some aspects of the medical consideration of exposure to pesticides. The summary shows the following needs:

1. Surevillance over the use of pesticides by teams of two, one trained in the practical aspects of the use of pesticides and the other in the health aspects.
2. Obligatory reporting of illness due to poisoning by pesticides and systematic study of all necropsies to delineate the deceased's exposure to, and accumulation of, pesticides.
3. Thorough physical examination and indoctrination in safe procedures for working with pesticides of all who regularly contact pesticides.
4. Wide dissemination of ways whereby individuals can recognize that they are being affected adversely by pesticides and indoctrination with the fact that pesticides are inherently dangerous materials, which must be handled with respect.
5. Wide dissemination of knowledge on first-aid procedures in poisoning by pesticides.
6. Complete knowledge of the mechanisms of action of all pesticides in use, including effects on organizing embryos, developing fetuses and growing young as well as on adults.
7. Specific treatments for intoxications by all types of pesticides, with clear indications of differences in response to therapy of poisonings by specific pesticides within each group.
8. Better diagnostic procedures for recognizing poisoning by specific types of pesticides, including improved and simpler analytical methods, biochemical tests and clinical examination procedures.

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CHAPTER 5

Carcinogenicity of Pesticides

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CARCINOGENICITY OF PESTICIDES

SUMMARY

The Technical Panel on Carcinogenesis examined the available reports on tests of tumorigenicity conducted on about 100 pesticidal chemicals. From these reports it separated those which did not provide information sufficient for it to reach a judgment as to tumorigenicity. The remaining reports provided a basis for assigning each of 79 pesticides to one of three major groups:

A. Those judged "not positive for tumorigenicity."

B. Those judged "positive for tumorigenicity."

C. Those for which the evidence was considered insufficient for judgment.

All other pesticides fall into Category D: "Available information insufficient to justify any comment."

On the basis of its conclusions of tumorigenicity of specific pesticides the Panel has recommended the following actions:

Group A pesticides.—No action be taken to alter current practices.

Group B pesticides.—Exposure of human beings be minimized and use of these pesticides restricted to those purposes for which there are judged to be advantages to human health which outweigh the potential hazard of carcinogenicity.

Group C pesticides.—Graded priorities for additional testing based upon findings recorded in this document plus other indications for concern, coupled with suggestions for similarly graded reductions of human exposure to some of these pesticides.

Group D pesticides.—Appointment of a body of scientists to take up a continuing search of all sources of reports on tests of tumorigenicity of pesticides, and to assign the remaining pesticides to appropriate categories of priority for additional studies and regulatory action. In this regard, exemption of selected pesticides from requirements for testing, where based upon a "grandfather clause," is regarded as unsound.

The Panel has also offered a number of general recommendations as to:

1. Regulation of use practices;

2. Routine testing for tumorigenicity for regulatory purposes, as well as augmentation of methodologic and other research;

3. Availability of information on pesticides testing results;

4. Legislative needs.

One member of the panel of eight dissented from several of the above-stated opinions. The dissenting opinion is stated in full on pages 483–488 of this document.

INTRODUCTION

This Technical Panel on Carcinogenesis was charged with responsibility for interpreting available reports on the carcinogenicity of pesticides, with the purpose of estimating, insofar as possible, the carcinogenic hazard which these substances might pose to human health and the development of recommended courses of action based on these findings.

Reports on the tumorigenicity testing of pesticides exist principally in three sources: the general scientific literature, the files of the Federal regulatory agencies, and the files of the pesticides manufacturing industry. The general scientific literature contains only a fraction of the reports of tests which must have been conducted. Unpublished reports in the files of industry and of Federal regulatory agencies are less accessible than the general scientific literature. Of the pesticides listed in the report by Neumeyer et al. (*1*), studies of carcinogenicity have been reported in the general scientific literature for but about one-fifth, and this figure includes those pesticides reported for the first time in the June 1969 issue of the *Journal of the National Cancer Institute* (*2*). The Food and Drug Administration made available reports in response to specific requests. Industry's files were not examined. Accordingly, the Panel cannot state the completeness of its information. It appears probable that some of the carcinogenicity testing which has resulted in negative findings has not come to the attention of the Panel. Such negative data are not readily accepted for publication by most scientific journals.

The quality of evidence in some of the reports was another source of difficulty. Many of the reports, published and unpublished, failed to provide enough data to permit judgments as to the safety of some pesticides. Details of the experiments (numbers of animals, description of controls, duration of the experiments) were sometimes missing.[1]

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[1] Information on the purity of the chemicals tested was not uniformly available to the Panel except in the instance of the study conducted at the Bionetics Research Laboratories for the National Cancer Institute. While the report of this study (*2*) does not contain this information, it may be obtained by writing to the Research Information Branch, Office of the Director, National Cancer Institute, National Institutes of Health, Bethesda, Md. 20014.

The conclusions of the Panel, noted below, are based on examination of the available data on experience with laboratory animal systems. The Panel is fully aware of the difficulties in extrapolating from laboratory experience with experimental animals to the human situation. However, the epidemiologic approach to study of a near ubiquitous environmental contaminant, such as certain persistent pesticides, is equally complex. Very little acceptable epidemiologic data exist concerning pesticides in relation to chronic disease in man. These topics are addressed more completely in later sections of this report.

During its discussions, the Panel frequently was reminded of the complexity of the total environment, not only in respect to pesticides but also to other chemicals, pharmaceuticals, and biological agents affecting the human organism. Attention is drawn to this matter because intelligent management of pesticidal agents requires a comprehensive understanding, with due attention to all sources of human exposure. Germane to this issue is the potential for interaction among pesticides, of pesticides with other chemicals, and suspected factors in cancer causation. The Panel recognized the complexity of the potential interactions in carcinogenesis and the extreme difficulty of the task of unraveling them. In this context, the sparseness of reports on the topic of interactions among pesticides in carcinogenesis indicates a need for further attention to this aspect of the problem.

In developing its recommendations, the Panel was cognizant of the uses and benefits of pesticides. The Panel concluded that these matters were beyond its expertise and responsibility, and were properly the responsibility of other committees of the Commission. It developed its recommendations on the basis of issues of carcinogenesis and public health only while recognizing that the recommendations of the Commission as a whole with respect to specific pesticides must be based upon a comprehensive consideration of all factors. In the case of DDT, for example, it appears probable that health benefits resulting from its judicious use in certain selected circumstances may exceed such hazards present in terms of carcinogenicity for man. This topic is addressed specifically in *Conclusions on Specific Pesticides* (pp. 470–472) and *General Conclusions* (p. 478).

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THE EVALUATION OF CARCINOGENIC HAZARDS

The Technical Panel on Carcinogenesis first considered factors involved in the evaluation of carcinogenic hazards of environmental agents, particularly pesticides.

An important mode of widespread human exposure to pesticides is through foods, and criteria established for the evaluation of carcinogenic hazards of food additives are applicable to pesticide residues. Additional criteria have to be applied for the control of other modes and sources of exposure by other routes (skin, inhalation, occupational exposure).

The Technical Panel on Carcinogenesis has reached the following positions:

1. The presence of carcinogenic substances (of both synthetic and natural origin) in food might be a significant factor in the occurrence of what is commonly referred to as "spontaneous" cancer in man and animals. Thus, an important objective in cancer prevention is the elimination, or reduction to a minimum achievable level, of all substances in the diet of man proven to be carcinogenic in either man or animal.

2. Since the effects of carcinogens on target tissues leading to tumor formation appear irreversible, with accumulation of effects over extended periods of exposure, *the reduction of exposure to carcinogenic substances to the lowest practicable level may be one of the most effective measures towards cancer prevention.*

3. Many different factors may influence dose-response in carcinogenesis in man and animals. Their complexity is such that no assuredly safe level for carcinogens in human food can be determined from experimental findings at the present time.

General principles and criteria for evaluation of carcinogenic hazards have been laid down by several expert committees convened in the last 15 years by scientific and public health agencies such as the World Health Organization, and Food and Agricultural Organization (*1*, *2*, *3*, *4*, *5*), the International Union Against Cancer (*6*, *7*), the National Academy of Sciences—National Research Council (*8*), the European Committee on Toxicity (Eurotox) (*9*, *10*), and the Food and Drug Administration Committee on Protocols for Safety Evaluation (*11*). Recommendations made in these reports express a remarkably unanimous view on the general principles and criteria to be followed for carcinogenesis safety evaluations, widely accepted in principle by the scientific comunity (*12*, *13*, *14*, *15*).

Testing procedures

General requirements for testing procedures have been outlined in the past (*3*, *7*, *8*, *11*). They can be summarized as follows:

1. *Identity of tested materials.*—Purity, stability, chemical and physical characteristics, and source of the sample to be tested should be established. Selection of materials to be tested should emphasize the substances and formulations to which human populations are exposed, which is to say, the materials should represent as closely as possible those with which the populations come into contact.

2. *Animals.*[2]—The species most practical for testing are rats, mice, and—as more recently shown—hamsters. Strains and colonies should be selected to provide adequate sensitivity to tumor induction, as revealed by positive control tests with known carcinogens. Their spontaneous tumor incidence should be recorded. Treatment should begin when the animals are young; the animals should be kept as free as possible from infectious diseases and parasites.

3. *Route of administration.*—Experience since 1959 has failed to validate the use of other than the oral route of administration for the *routine* examination of food additives: however other modes of administration should be used for studies which are intended to assess carcinogenicity of pesticides entering the human population by other routes.

4. *Number of animals.*—The number of animals in each test group should be sufficient throughout the tests to yield statistically significant results. It is important to stress that the detection of positive results in these bioassays depends on the development of tumor incidences significantly above the threshold of detectability for a given number of animals. Any carcinogenic effect below these levels will not be detected by the bioassays used. For example, when a zero incidence is observed in controls, negative results on 100 test animals at a high dose level only establish with a 95 percent confidence that the incidence does not exceed 3 in 100 under the conditions of the test.

5. *Maintenance and diets of the animals.*—All of these experimental conditions should be controlled, frequently monitored, and adequately reported.

6. *Pathologic evaluation.*—It is recognized as essential that a com-

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[2] The use of nonrodent species, recommended in the earlier reports, has now been substantially dropped. A suitable, practical nonrodent species would be useful but it is not available at this time. Carcinogenicity tests of food-borne pesticides require routine lifetime feeding of chemical compounds. While dogs have been employed for tests of carcinogenicity, with noteworthy success in selected cases (bladder carcinogenicity of aromatic amines), the requirement of lifetime feeding makes this species too expensive, in terms of time and funds, to be employed routinely.

plete post-mortem examination be performed on all animals by, or under the supervision of, scientists trained in pathology and familiar with the diseases of laboratory animals, particularly tumors. As a minimum, all organs showing suspect macroscopic lesions should be examined histologically. Certain organ systems require special techniques, such as distention of the urinary bladder with a fixative. Tumors should be classified according to recognized standards for carcinogenesis studies.

Evaluation of experimental animal tumors should be made by pathologists with full knowledge of the biological behavior of the tumors in the animal strain under study. All tumors that metastasize are considered malignant. However, many malignant animal tumors have little tendency to metastasize. The evaluation of their benign or malignant nature depends largely upon their histologic characteristics. In some cases it is not possible to diagnose, on the basis of morphologic grounds alone, whether a tumor is malignant or benign. In such cases, transplantation studies and knowledge of the life history of the tumor type under consideration may provide additional diagnostic help. However, the majority of the Panel recognizes that benign tumors may become malignant. The Panel is unaware of the existence of any chemical which is capable of inducing benign tumors only, which is to say, in the light of present knowledge, all tumorigens must be regarded as potential carcinogens. Thus, the majority of the Panel accepts tumorigenicity as an index of potential carinogenicity.

Interpretation of the results and validity of animal tests

Interpretation of results of bioassays on a test material includes consideration of the accuracy and significance of the experimental studies, i.e., experimental design, details of information on test materials, dosage, route of administration, metabolism, excretion and retention, controls (positive and negative), experimental animals and methods, survival, description and time of appearance of toxic and pathologic effects, number, type, and individual distribution of tumors.

Extrapolation from animal data to man.—The evaluation of carcinogenic hazards for man is based on a judgment of all available information: on bioassay, on toxicologic, metabolic, and pharmacologic studies, on the extent and route of exposure of man, and on epidemiologic studies. Each compound must be evaluated individually on the basis of all data on its use and effects, including whether residues may occur as a result of use of the particular compound, the nature of its metabolites in man, the storage or retention and excretion, etc. The position of this Panel is that the different qualitative and quantitative responses of various animal species, including man, to carcinogens make meaningful extrapolation from "no-effect" levels in dose-response studies in animals to man currently impossible. (See appendix C).

In brief summary: (1) Food additives and contaminants should only be permitted if evidence is provided of no carcinogenic effect after adequate long-term bioassays. The minimum requirements for such bioassays should include: Adequate numbers of animals of at least two species and both sexes with adequate positive and negative controls, subjected for their lifetime to the feeding of a suitable dose range of the test material, including doses considerably higher than would be present in food; (2) any substance which is shown conclusively to cause cancers in animals, when tested under these conditions, should be considered potentially carcinogenic for man and therefore not innocuous for human consumption. Tests which yield benign tumors will nevertheless raise the level of suspicion.

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CONCLUSIONS ON SPECIFIC PESTICIDES

The Panel has examined the data available to it on the tumorigenicity of pesticides in animals. These data have been reviewed on the basis of the principles stated in *The evaluation of carcinogenic hazards* of this chapter and the specific criteria which follow.

The Panel reviewed relevant reports and considered as acceptable data on bioassays only those which included the following information:

1. Number, strain, and sex of the animals used;
2. Biometrically adequate numbers of test animals and controls;
3. Adequate period of observation (at least 18 months in the case of rodents);
4. Evidence of pathologic examination of the animals and of classification of the observed tumors;
5. Definition of the materials tested, their mode of administration and dose;
6. Evidence that the dose levels tested included one near the maximum tolerated level, in the case of negative reports;
7. Evidence that the experimental conditions (e.g., selection of animals, age at start) provided a sufficient sensitivity to detect tumorigenic activity if present.

For each pesticide, the Panel reached one of the following judgments:

A. "*Not positive.*—Data acceptable, testing adequate, results *judged* negative for tumor induction in at least two species."

B. "*Positive.*—Data acceptable, testing adequate, results *judged* positive for tumor induction in one or more species and significant at the 0.01 level."

C. "*Evidence insufficient to judge.*—Additional data needed [3] according to the following priorities:

Priority group C1.—Tumor incidence significant at the 0.01 level but compound concluded to be less active than the mean of a group of positive controls employed in the screening operation.

Priority group C2.—Tumor incidence significant at the 0.02 level but compound concluded to be less active than a group of positive controls employed in the screening operation.

Priority group C3.—Tumor incidence increased in comparison with the negative controls but statistical significance was less than 0.02,[4] possibly because too few animals were observed.

Priority group C4.—Tumor incidence not elevated in adequate studies conducted in one species only but current guidelines require negative results in two animal species for judgments of negativity."

D.—"Information available is insufficient to justify any comment."

On the basis of the foregoing criteria the pesticides have been assigned to the following categories. In this respect, it should be noted that a judgment of "not positive" for tumor induction does not constitute assurance that the specific compound is entirely lacking in carcinogenic potential.

The recommendations relevant to each category constitute the best judgment of the Panel, based upon available data.

A.—Compounds judged "not positive" for tumor induction on the basis of tests conducted adequately in two or more species.

[Registered for use on food crops [1]]

Name	References	Species
Chlorpropham (CIPC)	1, 2	Rat, mouse.
Rotenone	2, 3	Mouse, rat.
Sevin (Carbaryl)	2, 4	Do.

[Registered but not for use on food crops [1]—None]

[1] This information with respect to pesticides in Categories A, B, C1 through C4 provided by the Food and Drug Administration.

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[3] In many cases this will require conduct of additional tests.

[4] A statistical significance of 0.02 is greater than 0.05 and less than 0.01.

It is recommended that no action be taken to alter current practices with respect to the pesticides in this category, "A".

B.—Compounds judged "positive" for tumor induction on the basis of tests conducted adequately in one or more species, the results being significant at the 0.01 level.

[Registered for use on food crops]

Name	References	Species
Aldrin	5	Mouse.
Aramite	2, 6	Mouse, dog.
Chlorobenzilate	2	Mouse.
p,p'-DDT	2, 7,[1] 8[1]	Do.
Dieldrin	5	Do.
Mirex	2	Do.
Strobane	2	Do.
Heptachlor[2]	9, 10	Do.
[Registered but not for use on food crops]		
Amitrole	2	Mouse.
Avadex (Diallate)	2	Do.
Bis(2-chloroethyl) ether	2	Do.
N-(2-hydroxyethyl)-hydrazine	2	Do.
PCNB	2	Do.

[1] Supportive evidence.

[2] Assigned to this group because a metabolic product, heptachlor epoxide, was judged positive for tumor induction, results being significant at the 0.01 level.

It is recommended that the exposure of human beings to pesticides in this category "B" be minimized and that use of these pesticides be restricted to those purposes for which there are judged to be advantages to human health which outweigh the potential hazard of carcinogenicity. In making this recommendation, the Panel has taken cognizance of the difficulties inherent in and resulting from its implementation. Accordingly, the following comments and observations are intended for consideration in interpreting this recommendation.

The case of DDT deserves special comment because of its prominent place in the pesticides armamentarium and because, in many respects, it is a good illustrative example. It is a substance which has contributed and could contribute important health benefits. It is also a substance which is widely used and with which we have now accumulated substantial experience and knowledge. Indeed, our knowledge of the biological effects of DDT, inadequate though it still is for wholly reliable judgment of safety, far outstrips that of any other insecticide of this type.

The evidence for the carcinogenicity of DDT in experimental animals is impressive and the Panel takes no exception to the conclusions as to DDT recorded in the JNCI report of the National Cancer Institute study. *This study has demonstrated that DDT increased the incidence of cancer in mice under the experimental conditions employed. However, this does not prove carcinogenicity for human beings at the very much lower levels to which they are actually exposed.*

Since tests with groups of laboratory animals comparable in size to large populations of humans are impractical, and because wide species differences exist, high levels of exposure are used. Whether or not this device is adequate for extrapolation from experimental results to the human situation remains very uncertain, for research on induced cancer is replete with examples of differences in responses of different species to various carcinogens. Furthermore the metabolism of many chemicals varies with dosage level.

Evaluation of human experience with DDT has revealed little if any evidence of long-term adverse health effects from its use. On the other hand, the observations of human experience have not been sufficient to eliminate the possibility that continued chronic exposure may slowly induce a low level of cancer in man (see appendix D).

Accordingly, with the evidence now in, DDT can be regarded neither as a proven danger as a carcinogen for man nor as an assuredly safe pesticide; suspicion has been aroused and it should be confirmed or dispelled.

In the resolution of this issue, mere repetition of the tests conducted at Bionetics Research Laboratories would be of only limited value. Of greater importance will be:

1. Studies conducted on several animal species,
2. A much more critical study of human experience,
3. Development of knowledge relative to comparative metabolism and factors controlling dose-response relationships which may reinforce and improve ability to extrapolate from the findings of animal studies to man,
4. Studies on very large groups of animals, at a range of dose levels including those comparable to human exposure,
5. Evaluation of interaction of or potentiation of DDT with or by other materials, and
6. Studies of the tumorigenicity of DDT administered to several successive generations of one or more animal species. (The Panel is aware that studies of this nature have been initiated.)

In planning and assessing this additional work, consideration should be given to such factors as exposure to other similar or coacting materials and other routes of exposure.

It is the opinion of the Panel that, in view of the important benefits arising from the use of DDT, the current evidence is not sufficient to justify unqualified banning of the insecticide. On the contrary, the benefits from its use in the control of a number of insect-borne diseases, such as malaria and typhus, probably outweigh the possible dangers of carcinogenesis from its use.

However, suspicion of danger is present and prudence requires that: (1) Usage be reduced by restricting it to high-priority applications until more decisive information can be developed; (2) meanwhile the issue must be clarified, as noted above, while (3) at the same time alternates for DDT should be sought. In the latter endeavor a warning must be urgently sounded that a replacement not be accepted which, being new and poorly studied, may yet, in fact, be more dangerous than DDT.

The high priority uses which should continue include such examples as the control of malaria and typhus where these are major public health problems. Normal agricultural and nonessential mosquito control usage should be abandoned as soon as possible.

But, discontinuance of such lower priority DDT usage will by no means immediately eliminate DDT or other persistent pesticides as contaminants of our foodstuffs. DDT is now a contaminant of crop-producing soils and of water in many parts of the country. Although this contamination will decrease with time, and realistic means for acceleration of its disappearance must be sought, trace contamination of the American diet with DDT will continue. With the presently high levels of sensitivity of analytical detection it can be anticipated that such trace contamination will be detectable in a very significant part of the foods making up the American diet for some years to come. Accordingly, strict interpretation of present legislation would present, through rejection of a major part of our food sources, a far worse health hazard than the uncertain carcinogenic risk of these trace amounts. In short, we should:

(1) Now reduce food residues through elimination of the use of DDT and DDD in food production,

(2) Reduce contamination of soils and water insofar as possible,

(3) Not deny a major food need to our country because of the detection of trace quantities of DDT resulting from previous use of this pesticide.

C.—Compounds on which additional data are needed.—Pesticides are listed in groups which are arranged according to priorities for additional testing. These priorities have been established on the basis of the Panel's judgment of significance of the available data indicating tumorigenic potential; additional priorities could be established on the extent of use of the compounds or on their structural relationships to known chemical carcinogens.

Priority group C1.—The following compounds yielded an increased tumor incidence significant at the 0.01 level but were considered less tumorigenic than the mean of a group of positive controls. These compounds have first priority for additional testing.

[Registered for use on food crops]

Name	Reference	Species
p,p'.-DDD	2	Mouse.
Monuron	2	Do.
Perthane	2	Do.
Piperonyl butoxide	2	Do.
Piperonyl sulfoxide	2	Do.

[Registered but not for use on food crops]

Name	Reference	Species
Azobenzene	2	Mouse.
CCC	2	Do.
Chloranil	2	Do.
Cyanamide	2	Do.
Vancide BL	2	Do.
Zectran	2	Do.

Priority group C2.—The following compounds yielded an increased tumor incidence significant at the 0.02 level. Similarly, they were concluded to be less tumorigenic than the mean of the same group of positive controls. These compounds have second priority for additional testing.

[Registered for use on food crops]

Name	References	Species.
Biphenyl	2	Mouse.
Captan	2	Do.
2,6-Dichloro-4-nitroaniline [1]	2	Do.
Gibberellic Acid	2	Do.
2-Mercaptobenzothiazole (Captax)	2	Do.
Ovex (Chlorfenson)	2	Do.

[Registered but not for use on food crops]

Name	References	Species.
Genite-R99	2	Mouse.
IPC (Propham)	2	Do.

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[1] Active ingredient in the formulation sold as Botran. See also Botran in priority group C4.

Priority group C3.—The following compounds yielded an increased tumor incidence in comparison with the negative controls but the level of significance was less than 0.02, possibly because too few animals were observed. These compounds have third priority for additional testing.

[Registered for use on food crops]

Name	References	Species
α-(2,4-Dichlorophenoxy) propionic acid	2	Mouse.
2-(2,4-DP)	2	Do.
2,4-D Isopropyl ester	2	Do.
n-Propyl isome	2	Do.
Pyrethrin	11	Rat.
Zineb	12, 13	Do.

[Registered but not for use on food crops]

Name	References	Species
1-Naphthalene acetamide	2	Mouse.
2-(2,4,5-Trichlorophenoxy) propionic acid	2	Do.

Triphenyltin acetate belongs in this group but is not registered as a pesticide. However, Triphenyltin hydroxide is a pesticide registered for use on both food and nonfood items. Triphenyltin hydroxide was not tested for carcinogenicity.

Name	Reference	Species
Triphenyltin acetate	2	Mouse.

Priority group C4.—The following compounds were tested appropriately in one species only and judged not positive in that species. However, current guidelines for testing require negativity in two species. These compounds have fourth priority for additional testing.

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[Registered for use on food crops]

Name	References	Species
Atrazine	2	Mouse.
Botran [1]	2	Do.
Butacide [2]	2	Do.
2,4-D	2	Do.
2,4-D Butyl ester	2	Do.
2,4-D Isooctyl ester	2	Do.
Dehydroacetic acid	2	Do.
Dichlone	2	Do.
Diuron	2	Do.
Dodine	2	Do.
Orthophenylphenol	2	Do.
Endosulfan	14, 15	Rat.
Ferbam	2	Mouse.
Folpet	2	Do.
Glyodin	16	Rat.
Maleic hydrazide	2	Mouse.
Maneb	2	Do.
Methoxychlor	17	Rat.
Methyl Zimate (Ziram)	2	Mouse.
Nabam	2	Do.
Phenothiazine	2	Do.
Planofix: N.A.A	2	Do.
Propazine	2	Do.
Simazine	2	Do.
Tetradifon	2	Do.
Thiuram (Thiram)	2	Do.
Tillam-6-E	2	Do.

[Registered but not for use on food crops]

Name	References	Species
ANTU	2	Mouse.
Cacodylic acid	2	Do.
Copper 8-Hydroxyquinoline	2	Do.
Dicryl	2	Do.
Diphenatrile	2	Do.
Dowcide-7 [3]	2	Do.
Hercules 7531 (Norea)	2	Do.
Isolan	2	Do.
Karathane	2	Do.
Pma; Phenylmercuric acetate	2	Do.
2-Sec.-butyl-4, 6-dinitrophenol	2	Do.
2,4,5-T	2	Do.

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[1] Botran. Compare with active ingredient 2-6-Dichloro-4-Nitroaniline which has been assigned to priority group C2.

[2] Butacide. Compare with active ingredient piperonyl butoxide which has been assigned to priority group C1.

[3] While Dowcide-7 is registered for nonfood uses, its use in food containers and packaging materials is permitted under registration. Wooden fruit (berry) boxes may contain up to 50 ppm.

It is recommended that:

1. Pesticides in *Priority Group C1* be immediately subjected to additional testing for tumorigenicity and that human exposure to all sources of these pesticides be restricted to a minimal level until the results of such additional testing permit development of judgments as to probable hazard to man.

2. Exposure of the general population to food sources of the pesticides in *Priority Groups C2 and C3* be reduced as much as possible pending the conduct and evaluation of additional testing of tumorigenicity. Exposure of occupational groups should be minimized in respect to all modes of contact with these pesticides pending completion of such studies.

3. Pesticides in *Priority Group C4* be the subject of a thorough search for additional information in the very near future, and such additional studies as may then be needed to meet the criteria for judgments of safety be conducted. Until further search for information reveals a cause for suspicion of potential carcinogenicity, no interim action to modify current use practices in this Priority Group is indicated.

(It should be noted that the basis for the differences in priorities for Groups C1, C2, and C3 is the tumorigenic potency of the compounds as revealed in the animal test systems.)

D.—Available information insufficient to justify comment in any respect.

All pesticides not listed in one of the above groups (A, B, C1, C2, C3, C4) are assigned to this category (D) until reports can be located and appropriate judgments made, as indicated below.

It is recommended that responsibility for continuing the search for and evaluation of reports on pesticides in this category be assigned to a duly appointed body of scientists which would have full access to the files of Federal agencies concerned with regulation of pesticides use, and which would seek the cooperation of the chemical industry to obtain such additional information as its members may have accumulated on this topic. Following completion of such search and evaluation, it should be the responsibility of the appointed body of scientists to assign further priorities for study and regulatory action with respect to each pesticide.

It is further recommended that the existing agencies of the Federal Government which are concerned with the regulation of pesticides use take immediate steps to require orderly testing of tumorigencity of pesticides listed in groups C1 through C4 above. The concept of a "grandfather clause" exemption from testing may be justifiable in respect to the hazards of inducing reversible disease. It is not justifiable, however, in respect to the hazards of inducing diseases which are not reversible, especially where the latent period is very long and the hazard may go unrecognized. Cancer is one of those diseases.

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General Conclusions

1. Use practices—regulation in respect to potential hazards of carcinogenicity

Although cancer is only one of many possible toxic responses to noxious chemicals in food, certain characteristics of this disease response justify its separate consideration. These characteristics include:

a. A generally slow, prolonged, and covert development;

b. Essential irreversibility of the lesions once the malignancy develops; and

c. The present uncertainty in making reliable predictions of cancer hazard for man by laboratory tests.

Accordingly, it is recommended that the use of any amount of a potential carcinogenic[5] pesticide, such that a food residue results, be allowed only if:

a. Health values to the public are such that banning the use would itself constitute a more certain detriment to public health, and if

b. No adequately proven noncarcinogenic alternative is available.

In this regard, the recommendations for action on pesticides in Group B (Conclusions on Specific Pesticides, pages 470–472 of this document) are in need of urgent attention.

When the complete ban of use of a carcinogenic[5] pesticide proves impossible for these reasons, its use should be reduced to the minimum possible extent compatible with its benefits for public health, and noncarcinogenic substitutes should be actively sought. The public should be informed of the potential hazards resulting from exposure to the compound.

In seeking substitutes for potentially carcinogenic pesticides preference should be given to the less persistent candidates, provided they do not present other more immediate and serious hazards. All other factors being equal, the degradable pesticides are regarded as less hazardous in respect to carcinogenicity than the persistent pesticides.

2. Hazards (Carcinogenicity) evaluation—routine testing practices for regulatory purposes

Some pesticides which leave residues in foods have been tested for tumorigenicity; others, to the knowledge of the Panel, have not. The methods employed in the studies examined by the Panel vary substantially in their quality and reliability; some of the tests conducted must be concluded to be inadequate.

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[5] Carcinogenic to animal species but not proven to be carcinogenic for man.

Testing of chemicals for tumorigenicity by present methods is expensive and slow. There is a need for an augmented effort to develop more efficient systems of predicting human response to chemical substances in respect to carcinogenicity. Such systems must be examined for relevance and reliability by comparing their predictive performances with actual human experience. Judgment as to potential carcinogenic hazard must take into account not only the laboratory tests but all other relevant information, including experience of humans exposed to these or related compounds. Existing epidemiologic data in respect to carcinogenicity of pesticides are scant. The observations of which the Panel is aware have been limited to small groups of people who have been observed for relatively short segments of the human life span. Such studies do not provide a basis for final assessment as to safety or carcinogenic hazard of pesticides. (The needs for epidemiologic studies are discussed more fully in appendix D.) Once tolerances are established for individual pesticides and these pesticides are approved for use, surveillance of human populations should be undertaken on a continuing basis to verify earlier predictions as to safety.

Accordingly, it is recommended that:

a. All existing pesticides whose use may leave residues on consumable food be subjected to tumorigenicity tests performed according to the guidelines discussed in *The Evaluation of Carcinogenic Hazards* of this chapter.

b. Approval to use new pesticides which may leave residues on consumable food be conditional upon *prior* testing for tumorigenicity according to the guidelines discussed in *The Evaluation of Carcinogenic Hazards* of this chapter.

c. Development of priorities for testing of existing pesticides for tumorigenicity be based upon such considerations as chemical structure (suggestive of tumorigenic potential), extent and patterns of usage, and levels of residue left in consumable foods. In developing priorities for additional testing, the finding of tumorigenicity in prior studies should be given close attention. On this basis, the pesticides listed in priority group C1 of this document merit prompt action.

d. Research to develop more efficient laboratory methods of prediction of carcinogenicity of pesticides for man be augmented substantially.

e. The predictive reliability of laboratory methods, both existing and those to be developed, be corroborated as completely as possible by comparison of the predictions with human experience.

f. A surveillance network of epidemiologic studies of carcinogenicity of pesticides in man be established and that special attention be directed to populations experiencing high levels of pesticide exposure (see appendix D of this chapter).

3. Availability of information

The reports of tests of the tumorigenicity of pesticides are scattered among several sources and have been difficult to assemble for review. In the interest of efficiency and effectiveness of future efforts there is a need for development of an information system capable of responding promptly and completely to individual requests.

Accordingly, it is recommended that reports of tumorigenicity testing be made available on request by the establishment of a Government clearinghouse and repository for the complete test data. Th Government agency should then be required to publish a listing which will identify these reports, thus making them accessible for review. Included should be the scientific basis on which decisions were made to register a pesticide and to establish tolerances. *It is further recommended that* there be established a standing committee of advisers to the Secretary to oversee the operation of this facility and to insure continuing re-evaluation of all extant information on the health hazards of pesticides, and other related matters.

4. Legislative needs

The deliberations of the Panel have disclosed disturbing gaps and disparities in the present regulatory laws aimed at insuring safety of the public (and occupational groups) from chemical exposures. Thus, although the pesticide and the food additive legislation provide for reasonably detailed and thorough testing and safety assessments, the Hazardous Substances Act covers only some household products and deals very inadequately with long-term effects, such as cancer. Some materials are inadequately covered by existing legislation and, for all practical purposes, receive cursory examination or, indeed, may escape review entirely. In addition, the present regulatory provisions give either inadequate or no consideration to simultaneous human exposure through several routes, an example being the inhalation of pesticides by the home gardener using spray cans of the same or similarly acting compounds and ingesting pesticides in food and water. Finally, imposition of a zero tolerance, which conceivably could occur as the result of an interpretation of the Delaney clause,[6] could, in the example of DDT, present a major national nutritional problem. New legislation should take into account the need for reconciliation of public interests, the implications of advancing technology which has increased the sensitivity of chemical detection more than one thousandfold in the past 15 years, and the problems of safety evaluation of chemicals as discussed in this document.

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[6] The Delaney clause of the Food Additives Amendment of 1958 to the Federal Food, Drug, and Cosmetic Act, Public Law 85–929, 85th Cong., H.R. 13254, Sept. 6, 1958.

Accordingly, it is recommended that legislation aimed at protecting the public health in respect to pesticides and other foreign chemicals be completely reviewed and appropriately revised to minimize hazard, taking due regard of the patterns of usage and the modes of entry of the chemicals, while preserving the beneficial uses of the chemicals as much as possible.

APPENDIXES

APPENDIX A

A review of the National Cancer Institute study [1]

1. Majority opinion.—In recognition of the prominent position which the National Cancer Institute Study (*1*) occupies in the spectrum of efforts to characterize the carcinogenicity of pesticidal chemicals, the Panel believes it appropriate to address a few remarks to the methods employed in this study.

At the outset, it should be pointed out that the pesticides selected for study were not chosen in a random fashion. Rather, they were chosen on the basis of three criteria: (*a*) evidence of toxicity, described in the literature, suggesting potential hazards to man; (*b*) widespread use of the chemicals; or (*c*) chemical structure suggesting possible carcinogenicity.

The methods selected by the investigators sought deliberately to maximize the probability of discovery of carcinogenic potential possessed by the chemicals selected for study. For this purpose, very large doses were employed, in most cases, and administration of the chemical to the mice was commenced as early as possible after birth and continued for the lifetime of the animals.[2]

The test animals were chosen to combine identified advantages in their parent strains and thus yield a maximum amount of information as to tumorigenicity of the test compounds. The numbers of animals per group were sufficiently large to provide a sound basis for statistical analysis of the results. Both negative and positive control groups were employed; their numbers and varieties were fully adequate to the requirements of the study. Although randomization of litter mates to groups could not be accomplished because the experiments were initiated in the preweaning period, a number of analyses of the results have satisfactorily excluded significant litter effect on those results.

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[1] Conducted at Bionetics Research Laboratories under contract Nos. PH43-64-57 and PH43-67-735.

[2] In this case, lifetime equates with the period of observation, which was 18 months.

Periods of observation of the test and control animals varied to an insignificant degree. It is the opinion of the Panel that this variation is not a basis for explaining the differences between the test and control groups.

The end point employed in the study was tumors. This term was used to include the benign and the malignant neoplasms and those neoplasms whose malignancy could not be ascertained on the basis of histomorphology alone. Difficulty in diagnosing malignancy on the basis of histomorphology alone was encountered in respect to the tumors of the liver and lung; it was not encountered in respect to other tumors such as the lymphomata. This use of the term "tumor," in the opinion of the panel, is both useful and admissible for the purposes of quantitating chemical carcinogenicity in animals. Its admissibility is based upon two facts: (*a*) No adequately tested chemical has been found to produce only benign neoplasms and, (*b*) a substantial percentage of benign-appearing tumors in mice has been demonstrated ultimately to eventuate in cancer.

A major test of the validity of a system employed as a measure of the tumorigenicity of specific pesticidal chemicals in animals is the consistency of its results with what has been found in other animal systems which have been and continue to be accepted as valid. In this study the test system responded to the negative and the positive controls in the appropriate manner. Among the positive controls, the degrees of potency revealed in this study were in agreement with earlier findings in other systems.

Several questions arise. The most prominent is the relevance of animal responses to those of human beings in respect to any given kind of noxious agent. The next question is the relevance of findings experimentally induced in animals to diseases occurring in men, not only in terms of the general conditions of exposure but also in terms of the exposure levels. These questions are germane not only to the National Cancer Institute study but also to all laboratory tests of carcinogenicity of specific chemical pesticides. As is indicated elsewhere in this document, uncertainties as to relevance and extrapolation are a cause for concern and caution in interpretation of results. It should be noted, however, that a remarkable degree of concurrence has been found to exist between chemical carcinogenesis in animals and that in man where it has been studied closely.

In brief summary, the National Cancer Institute study should be regarded as a fine-mesh screen designed to identify as many as possible of the carcinogens submitted to it. It has performed this task with considerable success. It should be emphasized, however, that this system detects a capacity for injury of the experimental animal; it is not purported to define "no effect levels," the existence of which is debated. Neither is it purported to predict the response of human beings to the noxious agents, either at the dose levels actually administered to the experimental animals, or at the dose levels commonly encountered by human populations.

2. Dissenting opinion.—(Mr. Carrol Weil's critique of the National Cancer Institute study, and his other comments dissenting from the opinions and judgments of the majority of the Panel.[3])

> The following are some statements in reference to the experimental design and subsequent analysis of data of the Bionetics study:
>
> I. *Dosage regimen.*—The concentrations in the diet employed in the BRL studies were large by almost any standard but they were not actually the maximum tolerated. Because the maximum tolerated dosage was determined in very young animals and dosages were not increased in a constant relation to the weights or surface areas of the individual mice as they grew, the dosages administered actually receded from the maximum tolerated by the young mice by 3 to 4 fold. It is possible therefore that some of the chemicals studied might have been capable of inducing cancer had larger doses been employed.
>
> II. *Route and time of administration.*—The wisdom of administration of the test substances to animals during the preweaning period, especially by intubation, has been questioned. The basis for the concern expressed has been the presumed extraordinarily high peak dose of the material reaching the target organs in a short period of time each day of administration, which is to say, extraordinarily high in contrast with those reaching the target organs following administrations via the diet. The major objection to this former method of administration is that it is not comparable to the human situation and might have been the reason for results which would not, if the materials were only fed in the

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[3] Each of the issues raised by Mr. Weil was discussed in the panel sessions and eventually resolved in the manner presented in the main body of this report. In particular, following the original statement of these criticisms by Mr. Weil, which appear in secs. IV and V of his dissenting opinion, the data were reexamined on a litter basis, in keeping with the Epstein-Mantel approach, rather than on the single-animal-basis employed in the Journal of the National Cancer Institute report. *All compounds which had been judged positive for tumor induction (significant at the 0.01 level, or stronger) remained positive.*

diets, have occurred. The implication has been that the experimenters have taken undue advantage of a period of peculiar and excessive responsiveness of the test animal in a manner which has no relevance to the potential hazard of the materials in the human situation.

The newborn and very young mouse differ from the adult in many ways. Toth, Magee, and Shubik (*1*) stated that "the activities of certain liver enzymes in newborn mice were shown to be five times smaller than in adults. Moreover, it was demonstrated that DMBA persists for a longer period of time in newborns than in adult mice. In accord with this finding, the catabolic rate of urethan was reported considerably higher in adult mice than in younger and newborn animals." Kaye (*2*) stated that "the relationship between the greater retention of urethan by young than by old mice and the greater response of younger mice to carcinogenic action of urethan suggests that the length of time urethan remains in the body is a critical factor in determining tumor yield."

Giving doses by repeated *oral intubation* also eliminates possible maternal metabolism of the material. Because of this possible conversion into a less carcinogenic metabolite, the high daily peaks which would not occur if the materials were fed in the diets and the fact that intubation into the young without maternal passage is foreign to any human hazard route, this method of administration is not recommended for any future studies. (*1*) Toth, B., Magee, P. N., and Shubik, P.: Carcinogenesis study with dimethylnitrosamine administered orally to adult and subcutaneously to newborn BALB/c mice. *Cancer Research 24:* 1712–1722, 1964. (*2*) Kaye, A. M.: A study of the relationship between the rate of ethyl carbamate (urethan) metabolism and urethan carcinogenesis. *Cancer Research 20:* 237–241, 1960.

III. *Age at start.*—Some aspects of the use of 7-day-old mice were referred to above. It can be argued that a parallel could exist with human infants or with cow's milk *only if* a pesticide had been included in the diet of the test animal, *not* when it was given by gastric intubation in the preweaning period.

IV. *Group size.*—The mice were not assigned to the various test groups at random. Entire litters were assigned to particular materials. Therefore, the number of experimental units for each material was not 18 animals in a given sex/strain group but the number of litters. Assuming a maximum of 12 pups per litter, six of each litter per group, a maximum of three of each sex per litter would have been used resulting in an N of approximately six instead of 18.

In studies such as this previously described by Epstein and Mantel (*Int. J. Cancer: 3*, p. 333, 1968), the following statement is made:

"In toxicity and carcinogenicity testing with neonates, it is necessary, for practical reasons, to treat all animals in each litter alike. This imposes limitations on the statistical consideration of all animals as individuals, according to conventional practice, without reference to possible litter influences."

This is what D. Mainland (in *Elementary Med. Statistics*, 2d ed., W. B. Saunders, 1963, pp. 59–60) terms "the error of wrong sampling units or spurious enlargement of samples or counting the same thing over again." He states, "it was not obvious to a distinguished worker in nutrition and dentistry who reported on the caries in 36,196 teeth in the mouths of 1,870 children. By examining about 20 teeth per child, the investigator had measured over and over again the same tendency (or resistance) to caries, but in the analysis each tooth was counted as if it gave an independent piece of information. The proper sampling units were children, and one way to express the information would be by the numbers of children with, and without, caries. A finer measure would be the number of carious teeth per child."

Other references by Epstein *et al* on the same subject are: *Nature 215*, p. 1389 (1967), "* * * tumors (hepatomas) were not randomly distributed between various litters."

Cancer Research 27: p. 1901 (1967), in reference to the hepatocarcinogenicity of griseofulvin "an apparent litter influence on the the distribution of hepatoma was noted."

Nathan Mantel discussed this in his talk "Some statistical viewpoints in the study of carcinogenesis," given at the International Symposium on Carcinogenesis and Carcinogen Testing, Boston, Mass., Nov. 8 and 9, 1967. He stated: "I will start with the premise that in work with newborn mice, all animals in a litter must ordinarily be treated alike. If this is the case, any data forthcoming from a carcinogenesis trial must be analyzed so as to take possible litter difference into account. To illustrate, suppose 10 of 100 treated mice develop a particular neoplasm while no such neoplasms occur among 100 control mice * * * highly significant * * *. But suppose that all 10 neoplasms had occurred in a single litter which had been assigned as a whole to the drug treatment group. The observed result should no longer be considered significant and the data would lend themselves to the interpretation that the particular litter which was going to get the neoplasms anyway had by chance, with 50-percent probability, been assigned to the treatment group."

V. *Randomization.*—The unit of comparison for statistical analysis between treated and control groups, must be litters, and not individual mice. There were approximately six litters per sex/strain and the reactions to these should have been compared to the concurrent controls. As the litters were not randomly assigned to the materials under test—compounds of high code numbers were started near the end of the program, e.g.—comparison to combined control groups is not proper. In fact, lack of randomization, to protect against unsuspected bias or nonrandom assignment of inherited characteristics, casts doubt on any statistical consideration.

Further, as the incidence in the subgroups differed (when mice were used as the unit), there is no valid biological or statistical reason to combine and compare the results of both sexes in one strain, or to combine and compare each sex *in both strains* or to combine and compare *grand totals* of *both sexes* in both strains.

VI. *Negative controls.*—All of the combined, control mice should not have been considered as N_c, to be used for comparison with all of the combined test mice, N_t, for any material because of the nonrandom assignment of entire litters to the materials, which were also not randomly started.

VII. *Distinction between compounds which "resulted in an elevated tumor incedence" and those which "require additional evaluation."*—For the following reasons, the materials reported to (*a*) have produced an elevated incidence of tumors in mice or (*b*) which require additional evaluation in the Bionetics study need additional testing before any of them can be considered to be a hazard to man or other animals:

(1) The mice were not assigned to the various test groups at random. Entire litters were assigned to particular materials. Therefore, the number of experimental units in each study was not 18 mice of each sex and strain, but the number of litters per subgroup.

(2) As the litters were not randomly assigned to the materials under test—compounds of high numbers were started near the end of the program, for example—comparison to combined control groups is not proper. In fact, lack of randomization, to pro-

tect against unsuspected bias or assignment of inherited tendencies, casts doubt on any valid statistical comparison.

(3) As the incidence in the subgroups differed, there is no biological reason to combine results of males and females in a strain, or to combine males or females in both strains, or to make and compare grand totals. Furthermore, as stated in (1) all comparisons should be only on litters as the units, not mice.

(4) The dosages were not maintained at a constant level—the proper method to use to assay the toxicity or carcinogenicity of a material.

(5) No assay was made of interim effects of the materials on tissues. All or most of the eventual effect may have resulted from the repeated intubation of large doses to the mice before weaning. This intubation route, with no maternal metabolic passage which might have detoxified the material, is completely foreign to any potential hazard route for humans. Therefore, concurrently dosed groups should have been run with the materials in the diets of the parent before the litters were weaned. Or, alternatively, this latter method should have been the *only* test method.

(6) While gastric papillomas might not have been accurately counted, they were recorded in vol. 1 of the report by the Bionetics Research Laboratories to the National Cancer Institute. They were, furthermore, listed on pp. 45, 46, and 47 of that report as being one of a "class of tumors requiring virtually no explanatory comment." The incidence of gastric papillomas was statistically significantly higher in the female B6C3F1 negative control mice than in eight of the 11 "experimental compounds which resulted in an elevated tumor incidence" in table 2 of the Innes, *et al.* publication. Therefore, some doubt is cast on the significance of the increase in incidence of hepatomas when the gastric papilloma incidence is concurrently decreased. Reasoning in (1) and (2) make both types of comparisons unreliable.

(7) As the hepatomas were generally not considered cancers, and as the incidence of lymphoma was quite low compared to the controls in each sex of each strain, and could have been the result of litter effect described in (*1*) and (*2*), none of the materials have been definitely "found to induce cancer when ingested by man or animal"—Section 409(c)3(A) of the Food, Drug and Cosmetic Act—in this study. Therefore, no action is required by the Secretary of the Department of Health, Education, and Welfare.

VIII. *The significance of the bionetics study.*—The Bionetics study was conceived as a screening test to evaluate a series of pesticides and industrial chemicals for potential tumorigenicity. As a screening test it indicated that (*1*) the positive controls increased the incidence of hepatomas (*2*) a number of compounds were found to also increase tumor incidences under the conditions of the test and (*3*) negative results were obtained on many compounds.

A large enough number of animals were used in each test to *expect* the results obtained to be significant statistically, although because randomization was not practical, the experimental unit should have been considered to be litters, rather than mice. However, because of the large number of chemicals and animals used, the newness of the test procedure, large differences between results obtained using different strains and sexes of mice, and the fact that the tests were carried out in one laboratory only, the work should be regarded as indicative but not conclusive. The results clearly point out the need for confirmatory tests preferably by a collaborative interlaboratory effort. Therefore, until the results of these are available, no sweeping statements should be made that certain pesticides are carcinogenic and/or should be banned. It would be in order to point out however that unnecessary human exposure to suspect compounds might be minimized until more evidence is available.

IX.—*Pathologic evaluation.*—The statement is made on page 466 that "The Panel is unaware of the existence of any chemical which is capable of inducing benign tumors only, which is to say, in light of present knowledge, all tumorigens must be regarded as potential carcinogens. Thus, the majority of the Panel accepts tumorigenicity as an index of carcinogenicity."

If only benign tumors result during the life-span of the experimental animals, as has happened in many studies, the above statement for these materials does not apply. If only benign tumors result, the material under test does not "induce cancer", which by definition of the term, is a malignant growth of tissues * * * associated with ill health and progressive emaciation.

APPENDIX B

Relationships between chemical structure and tumorigenicity in the National Cancer Institute study [1]

In general, the data obtained in the National Cancer Institute study show a consistent relationship between chemical structure and tumorigenicity.[2] Certain uniformities appear if one groups the compounds according to chemical structure and classifies them according to the tables in the report of the National Cancer Institute study (*1*):

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[1] Some of the chemicals listed in this appendix are not registered as pesticides; they are included in the list for illustrative purposes only.

1. compounds which resulted in elevated tumor incidence,
2. compounds which require additional evaluation, and
3. compounds which did not cause a significant increase in number of tumors[3] in the one animal species studied.

Chlorine-containing compounds.—A number of compounds which contain chlorine appear to be tumorigenic; however, chlorine substitution by itself does not impart tumorigenicity. Tumorigenicity is evidently regulated by other substituents in the molecule. The most important result is that all of those compounds in the experiment which are structurally related to DDT had ratings of 1 or 2.

p,p'-DDT	1
Chlorobenzilate	1
Perthane	2
o,p'-DDD	2
o,o'-DDD	2

Two other compounds containing chlorine atoms and two containing both chlorine atoms and nitro groups but unrelated to DDT also yielded positive or borderline results.

Mirex	1
Strobane	1
PCNB	1
Vancide PB	2

The second most important group of compounds which produced elevated tumor incidence contain chloroethyl or chloroallyl groups.

Aramite (positive control)	1
bis(2-chloroethyl)ether	1
Avadex (Diallate)	1
2-(chloroethyl)trimethylammonium chloride (CCC)	2

Several chlorophenols exhibited borderline activity, but an insufficient number of compounds of this class was included in the test to lend importance to this observation.

2,4,6-Trichlorophenol	2
2,2-Thiobis(4,6-dichlorophenol)	2
Pentachlorophenol	3

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[2] *Caution:* Notwithstanding the internal consistencies between chemical structure and tumorigenicity observed in this study, not enough is yet known about structure-function relationships to permit one to rely strongly upon similarities and dissimilarities of structure to predict tumorigenicity.

[3] At the 0.01 level of significance.

Compounds which contain nitrogen or pentavalent sulfur in addition to chlorine atoms were essentially non-tumorigenic in the test circumstances at the 0.01 level of significance.

Compound	Class	Rating
Monuron	urea	2
Diuron	do	3
Botran	amine	3
2,6-Dichloro-4-nitroaniline [1]	do	3
Dicryl	anilide	3
CIPC	carbamate	3
Endosulfan	sulfite	3
Ovex	sulfonate	3
Genite R–99	do	3
Tetradifon	sulfone	3

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[1] Same as Botran in chemical structure. Botran and this compound administered to separate experimental groups.

Also, many pesticides which are potential biological alkylating agents were inactive in the test circumstances. These include the following herbicides and fungicides:

Compound	Class	Rating
Chloranil (obsolete)	quinone	2
Dichlone	do	3
Captan	phthalimide	3
Folpet	do	3
Simazine	triazine	3
Atrazine	do	3

These results seem inconsistent with prior data since some biological alkylating agents have been shown to be carcinogens as well as carcinostats. However, if the compounds are too reactive chemically to be translocated, permeate cells, or be stored in tissues in sufficient amounts, then they may not be tumorigenic.

2,4-Dichlorophenoxyacetic acid (2,4-D) and five compounds closely related to it structurally were found to be nontumorigenic in the test circumstances.

Of the chlorine-containing compounds investigated, two out of seven main groups exhibited a significant degree of tumorigenicity. The results were internally consistent throughout.

Dithiocarbamates.—The dithiocarbamates can be classified into three main groups as follows:

Derivatives of diethylamine and bis(hydroxyethyl)amine

Dithiocarbamates:	Rating
selenium, diethyl	1
tellurium, diethyl	2
potassium, bis(2-hydroxyethyl)	1
sodium, diethyl	2
zinc, diethyl	3
cadmium, diethyl	3
Bid(diethylcarbamoyl) disulfide	2

Derivatives of dimethylamine

Dithiocarbamates:	Rating
lead	2
selenium	3
zinc	3
iron	3
copper	3
bismuth	3
dimethylammonium	3
Bis(dimethylcarbamoyl) disulfide	3
Bis(dimethylcarbamoyl) monosulfide	3

Ethylene(bis) dithiocarbamates

	Rating
zinc	3
manganese	3
sodium	3

Most of the diethylcarbamates were either clearly tumorigenic or borderline while the dimethyl analogues were inactive. Selenium, evidently, does not play a direct role in the activity of selenium diethyldithiocarbamate since the dimethyl analogue is inert. The three diethyl derivatives which would be expected to be assimilated readily are active while those including the insoluble heavy metal salts (Zn and Cd) are not.

The ethylene(bis) dithiocarbamates were inactive.

Carbamates and Thiocarbamates. The activity among the carbamates was not consistent. Ethyl carbamate and Avadex were tumorigenic. (The activity of the latter compound could possibly be ascribed to the chloroallyl group.) Zectran was borderline but the remainder of the carbamates did not produce significant effects.

Compound:	Rating
Ethyl carbamate	1
Avadex (Diallate)	1
Zectran	2
Sevin	3
IPC	3
CIPC	3
Isolan	3
Tillam -6-E	3

Ureas.—An insufficient number of urea derivatives was evaluated to give a clear picture of their activities. Ethylene thiourea, an oxidation product of nabam, was tumorigenic while monuron was borderline. Diuron did not produce a significant response.

Hydrazine derivatives.—Hydrazine has been reported previously (2) to produce hepatomas in mice. In this study N-(2-hydroxyethyl) hydrazine was found to be tumorigenic but maleic hydrazide was not. If free hydrazine were liberated enzymatically, tumorigenicity should ensue. It may be that animals differ in their abilities to liberate free hydrazine from maleic hydrazine. This could account for the conflicting reports which appear in the literature on the effects of maleic hydrazide.

Amines and anilides.—None of the amines and anilides tested exhibited statistically significant tumorigenicity at the 0.01 level. These include Dicryl, Botran and 2,6-dichloro-4-nitroaniline.

Methylenedioxyphenyl derivatives.—Compounds of this group were classified borderline or negative.

Compound:	*Rating*
Piperonyl butoxide	[1]2
Piperonyl sulfoxide	2
n-Propyl isomer	3
Butacide	[1]3

[1] Piperonyl butoxide was also tested as Butacide (piperonyl butoxide in a solvent vehicle). Because of the presence of the solvent, they were given at different gross dose levels. The difference in category assignment (2 versus 3) comes about because for piperonyl butoxide, 5 of 15 strain X male mice developed lymphomas (significantly greater than the negative controls—at the 1 percent level) and for Butacide, 3 of 16 animals (strain X, male mice) developed lymphomas. Three out of 16 was not significantly greater (at the 1 percent level) than the control levels (for the same strain and sex) while 5/15 was—hence the assignment of the two materials to two different categories. However, 5/15 is not significantly different from 3/16.

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(*2*) Biancifiori, C., Bucciarelli, E., Clayson, D. B., and Santilli, F. E.: Induction of hepatomas in CBA/Cb/Se mice by hydrazine sulphate and the lack of effect of croton oil on tumor induction in Balb/c/Cb/Se mice. *Brit. J. Cancer* 18: 543–550, 1964.

APPENDIX C

Animal studies of pesticides tumorigenicity: Dose-response relationships and extrapolation to "no-effect" levels

Dose-response studies are useful in demonstrating whether a response at a given dose with no gradations of response at lower doses is more suspect as "accidental" than a graduated response seen at graduated doses. Two possibilities in the biology of the response place restraints on the interpretations of the dose-response relationships, however:

1. Dose-spacing is very important. Given a very steep dose-response curve, too widely spaced doses could yield responses ranging from 100 percent at a given dose to zero percent at the next lower dose. This gives the appearance of an artifact when there is none. Similarly, a shallow dose-response with doses spaced too closely can give the appearance of no dose-response relationship, when, in fact one does exist.
2. The plateau phenomenon can obscure a dose-response relationship. That is, there may be a range of doses for which a dose-response does occur—up to some upper limit of response. At higher doses other toxic effects or other causes of death may intervene—and the tumorigenic action of the material may appear to be impaired. The plateau phenomenon is common in animal studies and has also been seen in humans. Notwithstanding these limitations in their interpretation, dose-response studies remain almost the only way to provide an estimate of a possible "safe" dose.

Reproduced below is part of a discussion of extrapolation to a "safe" dose, as presented in *The Report of the Subcommittee on Carcinogenesis of FDA Committee on Protocols For Safety Evaluation (1969)*. The concerned statisticians at the National Cancer Institute concur in these remarks.

"* * * the only practicable basis for estimating a safe dose is by extrapolation downwards from results obtained at some level well above the actual use level. But this extrapolation introduces serious uncertainties, which must be recognized if rational methods of safety evaluation are to be developed." [The basic problem is that extrapolation outside the range of observation must be based on a generally unverifiable assumption about the mathematical nature of the dose-response relationship near zero dosage. (Technical Panel on Carcinogenesis.)]

"It might be thought that the basis for such an extrapolation could be provided by observations in the observable range. To show how far from being the case this actually is, we give below three different dose-response curves, mathematically defined over a dosage range of 256 fold. All three have the same TD_{50} [1] and TD_{16} [1]. The first is a probit curve, the second a logistic curve, and the third the so-called one-particle curve.

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[1] TD_{50}=dose which results in tumors in 50 percent of the animals; TD_{16}=tumor dose for 16 percent of the animals, etc.

Expected percent of animals with tumors

Actual dose TD_{50}	Probit curve	Logistic curve	One-particle curve
16	98	96	100
8	93	92	99+
4	84	84	94
2	69	70	75
1	50	50	50
½	31	30	29
¼	16	16	16
⅛	7	8	8
1⁄16	2	4	4

It will be noted that below the TD_{50} the three curves differ by little and that in any experiment of practicable size (say less than several thousand animals) it would not be possible to conclude from the actual observations which one of the three best described the data. As shown below, however, the $TD_{.0001}$ (one in a million dose) and the $TD_{.000001}$ (one in one hundred million dose) obtained by extrapolation of these three curves differ markedly. Thus,

Extrapolated values of "safe" doses for three different dose-response curves describing observed responses in the 2 percent to 50 percent response range equally well

	Probit curve	Logistic curve	One-particle curve
TD_1	.040	.022	.014 4
$TD_{.1}$	.015 5	.003 15	.001 44
$TD_{.0001}$	.001 36	.000 009 8	.000 001 44
$TD_{.000001}$	.000 412	.000 000 16	.000 000 014
TD_1			
$TD_{.000001}$	100	100, 000	1, 000, 000

The one in one-hundred million dose, which Mantel and Bryan call the 'virtually' safe dose is one-hundredth the TD_1 using the probit curve, one-hundred thousandth using the logistic and one one-millionth using the one-particle curve. Thus, even with an experimentally well-determined TD_1, or dose at which $P_{max}=.01$, the 'virtually' safe dose is obtained by using a safety factor which can vary from 100 to 1,000,000 depending on the curve selected.

"The extreme unreliability of extrapolations outside the observable experimental range was the basic source of the failure of the early safety evaluation program for the Salk vaccine, even though the observed curve connecting log titer and inactivation time had some theoretical physical-chemical basis. Nor is the uncertainty attaching to an extrapolated value surprising, since even a relationship with as firm a molecular basis as Boyle's law breaks down at extremes of pressure and temperature. Clearly extrapolation from the observable range to a safe dose has many of the perplexities and imponderables of extrapolation from animal to man and it would be imprudent to place excessive reliance on mathematical sleight of hand, particularly when the dose-response curves used are largely empirical descriptions, lacking any theoretical physical or chemical basis.

"Nothing that has been said bears on whether a threshold dose does or does not exist. What does seem clear is that more fundamental experimentation than that of the usual toxicological dose-response investigation in intact animals is necessary to shed much light on the question."

The uncertainties in the extrapolation have led the FDA Subcommittee to the following conclusion.

> "Although it is possible in principle to estimate 'safe' levels of a carcinogen, uncertainties involved in downward extrapolation from test levels will usually result in permissible levels that are the practical equivalent of zero."

APPENDIX D

Studies of human populations

The relationship between pesticides and chronic disease, including cancer, has not been adequately studied in man. A review of available literature, including a MEDLARS search, reveals that most studies to date were done on small sample sizes, for short periods of time, and without adequate follow-up. This deficit points up the need for definitive epidemiologic studies on man to answer questions about variation within and among individuals, identification of "high-risk" and "low-risk" populations, relationship between exposure and risk, and establishment of human dose-response curves. A correlation between exposure to DDT (industrial and home use) and tissue concentration has been shown. However a correlation has not been established between tissue concentration and subsequent disease. Radomski *et al.* (*Food Cosmet. Toxic.*, 6:209–20, August 1968) have compared the organochlorine pesticide concentrations in fat and liver of 271 patients with known liver, brain, and other disease, against "control" levels found in an earlier autopsy study. They reported elevated pesticide concentrations associated with various diseases including a 2- to 3-fold increased concentration in patients with carcinomas of lung, stomach, rectum, pancreas, prostate, and bladder. No specific association was shown between increased concentration and any particular neoplastic disease. The authors postulated no cause-and-effect relationship. It should be noted that cachexia, accompanied by disturbances and shrinkage of fat deposits, may alter levels of fat-soluble pesticides in adipose and other tissues. Accordingly, it is possible that high concentrations of DDT in the remaining fat may not necessarily reflect the level of exposure of the individual.

To date there have been no large-scale, well-controlled, epidemiologic studies capable of revealing a negative or positive cause-and-effect relationship. At least one consequence of this lack of appropriate work is that there is also no evidence to prove that there is a safe threshold.

WHAT IS BEING DONE NOW

The majority of work in the field of pesticide data gathering on human exposure is being done by the Food and Drug Administration's Division of Pesticides in Chamblee, Ga. In 1949, the National Communicable Disease Center in Atlanta established a toxicology laboratory to begin studies on pesticides. In November, 1964, an Office of Pesticides was established to provide a mechanism for responding to a number of health-related recommendations which followed a study of pesticides by the Life Sciences Panel of the President's Science Advisory Committee. In August 1966, the office was transferred to Atlanta and renamed the pesticides program. It then included 12 community study locations. The program was transferred to the FDA on July 1, 1968.

The pesticides program, in addition to its community study projects, is currently conducting a Human Monitoring Survey, measuring adipose and serum pesticide levels and obtaining the following information: (1) name, (2) sex, (3) race, (4) age, (5) hospital and other identification, (6) up to three clinical diagnoses, and (7) up to three pathologic diagnoses. Specimens of tissue (or blood), removed routinely during surgery or autopsy, were obtained from collaborating pathologists. Demographic data were obtained from hospital admission sheets. Individual patients or families of deceased patients were not contacted. Due to the difficulty in getting accurate or complete information, data such as patient's occupation are omitted.

The data gathering has grown in an informal way. Between 70–80 pathologists around the United States were contacted initially. Later, attempts were made to improve national coverage by including pathologists from geographic areas initially omitted. No formal procedures exist to minimize bias in specimen collection. There are now 15 community study projects. The number of "occupationally exposed" individuals has been increased to 1,400. Numbers of controls have also been increased. The program over the last several years has reflected, as well as developed, improved technology for detecting and examining trace materials. Data handling has not developed satisfactorily. Each community study stores its own records, and there is no centralized data storage and retrieval system, though copies of records are available centrally.

From the Human Monitoring Survey data, the Pesticides Program is trying to obtain information concerning the incidence of various diseases (or mortality from several diseases) as related to pesticide levels. As of September 1969, the Program prepared a partial list of diseases (by ICDA classification) and the number of patients in the study with each disease. These data are not suitable for computing mortality rates. It is the intent of these studies to throw some light on causes of death, or diseases associated with high pesticide levels.

The major limitations of the current work are: (1) Incomplete demographic data collection, (2) non-representative population, (3) difficulties in computing rates of mortality, or disease incidence, (4) problems of identification of "high-risk" individuals, and (5) slow and uncoordinated data-processing procedures.

WHAT CAN BE DONE

A definitive, large-scale study is needed to examine the relationship between pesticides and disease. Such a study must be planned with a statistically sound sampling procedure including identification of low- versus high-exposure groups, multiple-tissue-per-patient examination, and adequate demographic data collection. Possible interactions of materials must be considered. Some of this is now being done on a limited scale. The data must be examined in various combinations in addition to the tabulation of incidence of disease by different tissue levels of pesticide. To identify increased incidence of rare diseases will require large sample sizes.

The sample sizes depend on the desired sensitivity of the study, the desired power of the study to reveal differences, if they exist, the incidence rate of the disease being studied, and the magnitude of change in disease occurrence that is meaningful to attempt to find. If one sought to find an increase in a relatively rare cancer, such as liver cancer, which animal studies indicate may be importantly affected by pesticides, then large numbers would be needed. (In 1966 there were approximately 2,000 reported cases of death due to primary liver cancer in the United States. This is a death rate of about 1/100,000.) At the 5-percent sensitivity level, close to 5,800,000 individuals would have to be studied to pick up a doubling of the death rate due to primary liver cancer. A sample large enough to pick up such an increase in liver cancer would, of course, be able to detect smaller increases in more common cancers. Consider cancer of the pancreas. At the 5-percent sensitivity level, a study of 5,800,000 individuals could reveal an increase of 33 percent in the death rate from cancer of the pancreas; to detect a doubling in the rate it would be necessary to study about 760,000 individuals. Major forms of cancer showing recent increases in incidence that might be related to pesticides include cancer of the colon, cancer of the bladder, and cancer of the pancreas.

By stretching out the study over several years, and by concentrating on age and occupation groups believed to be at high risk, the number of people who need to be followed can be reduced. Long term studies have the disadvantages of loss to follow-up, and protection against this needs to be built into them. This means increased initial sample size.

One other possibility exists that is worth considerable attention. That is to invert the process and, instead of looking at individuals and finding their diseases or causes of death, to look at causes of death, and find the characteristics of the individuals to whom these causes occur. This might be accomplished through a national death index.

A National Dealth Index—with all deaths within the United States tabulated by cause—would permit easier investigation of the pesticides problem. Where specific causes of death were suspected of possible association with pesticides, the Index would permit an identification of the individuals who died from these causes. Follow-up studies could reveal their occupations, possible exposures to pesticides, etc. Evidence of presumed clustering (in time and space) of deaths has stimulated epidemiologic investigations into possible etiologies of leukemias and lymphomas—with some profit. It might be worthwhile seeking information on clustering with respect to deaths presumed to be associated with pesticide exposure.

Without large-scale, well-planned, controlled epidemiologic studies, the true importance of the long-term effect of pesticides on the human population will be difficult to document with any accuracy. The Director of the Division of Community Studies, FDA, has remarked about the importance of "maintaining an overall *stable* program with *stature*." The Technical Panel on Carcinogenesis concurs.

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CHAPTER 6

Interactions

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INTERACTIONS

SUMMARY

Under experimental conditions, 3 kinds of interactions can readily be demonstrated. (a) in a few pairs of organophosphates one can block an enzyme (aliesterase) which normally degrades the other, and thus enhance its toxicity greatly; (b) most chlorinated hydrocarbons can, at fairly low concentrations, and especially if administered regularly, increase the level of the drug metabolizing system of liver microsomes. This increase produces a variety of effects, decreasing or increasing the toxicity to some pesticides which might be taken in later, but also altering the body's response to many drugs, and also changing the level of several circulating hormones; (c) piperonyl butoxide and related compounds, whose only commercial use is to improve the toxicity of pyrethroid insecticides, can at relatively high concentrations block the liver microsomal system mentioned above, and this blockade increases the toxicity of a variety of insecticides which are normally degraded by that system.

Although the above effects can be shown experimentally, the amounts of pesticide normally ingested, either by food intake or by those occupationally exposed, does not appear to be enough to create any hazardous interaction for compounds for which data are available. The amounts taken in by the general public are substantially less than enough to produce even detectable changes, and such just-detectable changes would have, in our opinion, no hazardous consequences. However, this opinion is necessarily based to a large extent upon data obtained with laboratory animals.

Although this report makes no recommendation specifically related to carcinogenesis, some consideration is given this aspect in appendix B.

There are two and possibly more conditions under which investigations are a cause for concern. One is when gross misuse or suicidal intent give rise to massive intakes. Under such conditions, the dangers of two compounds together will, for certain pairs of compounds, be greater than the sum of the dangers from each separately. The second is when individuals with a high occupational level of exposure to chlorinated hydrocarbon insecticides undergo therapy with certain drugs. Their response to these drugs may be quantitatively different from that anticipated.

CONCLUSIONS

1. Studies on the inhibitory action of anticholinesterase pesticides on plasma and liver aliesterases should be required as part of the routine toxicological evaluation studies because of the established importance of this inhibitory action as a cause of potentiation of toxicity.

2. Research should be conducted to establish the level of aliesterase inhibition by anticholinesterase pesticides that alters the rate of metabolism of ester drugs at therapeutic dosages.

3. Additional studies should be conducted to evaluate the significance of aliesterase inhibition in man, and especially liver aliesterase and amidase. If there is an increased use of organophosphorus insecticides and carbamate pesticides and a consequent increase in exposure of the population, especially those occupationally exposed, this increased health hazard must be monitored carefully to prevent an increase in the toxic action of other chemicals through inhibition of aliesterases. There is a need for methodology that will provide meaningful information on aliesterase levels in man.

4. The cumulative effects of anticholinesterase insecticides on cholinesterase and aliesterase activity of wildlife should be evaluated to aid in preservation of desirable species.

5. Tests for enzyme induction should be made a part of the required studies on all new pesticidal chemicals. *Quantitative* measurements of the potency of a pesticide as an enzyme inducer should be made using subacute or chronic tests unless separate experiments of a short-term type show that the pesticide is not capable of causing enzyme induction.

6. More research is needed to establish ways of predicting enzyme induction on a structure-activity basis.

7. More research is needed to establish qualitative and quantitative criteria to indicate similarities and differences in drug metabolism in various species. The extent to which the low levels of microsomal enzyme activity in extra-hepatic tissues such as lung, gastrointestinal tract, kidney, and adrenal cortex is induced by pesticides should be studied.

8. Additional research is needed on the development of practical quantitative methodology for measuring enzyme induction in animals and man. Consideration should be given to the development of model substrates with low toxicity whose metabolic products can be easily measured. When such methods are available monitoring studies should be conducted on individuals exposed in the manufacturing and application of pesticides.

9. Additional research designed to characterize the mechanism(s) and the significance of pesticide interactions should include the study of the effects of the total environment, i.e., chemical, physical, and social factors.

10. At sufficiently high levels, insecticide synergists inhibit the metabolism of insecticides. Information is needed a) on human intake of these synergists both by consumption of food in the normal diet and by inhalation exposure to aerosols as normally used, b) on the interactions resulting from intakes of this magnitude, first with experimental animals and subsequently with humans.

11. Further work should be encouraged on the effect of combinations of household detergents and pesticides and on storage of the latter compounds.

INTRODUCTION

The term *interaction* is commonly used to describe an increase or decrease in the biological activity of a chemical agent by the prior or simultaneous exposure to another chemical agent or other exogenous factor. Strictly additive toxic effects from chemicals having the same pharmacological actions are not classed as interactions, nor are those decreases in toxicity which are due to opposite pharmacological actions.

Interactions can be caused by several mechanisms. By far the most prominent mechanism responsible for known pesticide interactions is interference with the activity of drug metabolizing enzymes. Thus the ability of organic phosphate insecticides to inhibit esterases that detoxify other organic phosphates and some drugs represents one important type of interaction. Increases in the level of drug-metabolizing enzymes through induction by chemical agents represents another important mechanism. A third important mechanism involves inhibition of the activity of hepatic microsomal drug-metabolizing enzymes.

Some drugs (as distinct from pesticides) produce interactions by blocking excretion of other chemicals, some by competing for binding sites on plasma proteins, some through interference with intestinal absorption, some by blocking the transport of another chemical to its site of action, some by direct sensitization of the target site. The Committee has attempted to examine all types of potential interactions, but has found no documented cases of pesticides involved in these other types of interactions.

Therefore, the Committee focused attention on pesticide interactions that involve the three identified mechanisms. Careful consideration was given to the question of whether or not interactions could occur at the practical dose levels to which the population of the United

States is normally exposed. Additionally, the Committee has considered the same question in relation to individuals who are exposed to higher dosages through their occupation. When insufficient information was available the Committee made recommendations for further research.

PESTICIDE INTERACTIONS THROUGH INHIBITION OF ESTERASES

Pesticides having the same pharmacological action are generally additive in their toxicity and this has been recognized in regulations of the Food and Drug Administration (CFR 120.3) which prohibits the combined residues of more than one related compound on food commodities from exceeding the weighted average calculated from the individual tolerances.

Combined actions of an additive nature are readily predictable and understood in evaluation of potential hazards from pesticides. However, some pesticides exert more than an additive effect on the toxicity of other pesticides and present a combined health hazard in excess of that ordinarily expected. In 1957, it was reported that the simultaneous administration of two organic phosphate pesticides, EPN and malathion, gave acute toxic effects 50 times greater than a simple additive effect (*1*). It was also reported that subacute feeding of both compounds produced a ten-fold increase in toxicity as measured by cholinesterase inhibition. Protection of consumer health from such unexpected and unexplained "potentiation", as we shall call it, between organophosphorus insecticides was instituted by a change in FDA regulations to require the testing of each pesticide of this type in combination with each other organophosphorus insecticide (CFR 120.35). With the development of other cholinesterase inhibiting pesticides, namely the carbamates, this requirement was extended to include all combinations of organophosphorus pesticides and carbamates. Under the test procedures, if evidence of potentiation was observed in acute tests, subacute studies were required to define the level without toxic effect.

Partly because of this requirement, several other organophosphorus insecticides were uncovered which potentiate the toxicity of malathion and other organophosphorus insecticides (*2–8*). However, the majority of combinations studied did not cause significant toxicity beyond the expected additive action. Consequently, tolerances for cholinesterase inhibiting pesticides have been established with knowledge and evaluation of their potentiating effects in experimental animals. Confidence in this practice was obtained through administration of combinations of potentiating pairs at tolerance levels to human volunteers demonstrating no effect on cholinesterase (*7–9*).

More recently the biological mechanism for this type of potentiation of the toxicity of carboxyesters, like malathion, has become understood (*4, 10, 11, 12, 13, 14*). A few other interactions of lesser magnitude, not involving carboxyesters, have been reported whose mechanism is associated with competitive binding and other reactions (*8, 15, 16*). In the case of the more common and more marked cases of potentiation, it has been demonstrated that all organic phosphate and carbamate pesticides inhibit certain other esterases in addition to cholinesterase. Among these enzymes is a group referred to as aliesterases. One or more of these aliesterases rapidly hydrolyzes malathion to yield products which are devoid of anthicholinesterase activity, thus influencing detoxification of malathion. A metabolite of EPN possesses a greater inhibitory effect on aliesterase than on cholinesterase and when sufficient EPN is administered simultaneously or prior to malathion, inhibition of this aliesterase interferes with the normal detoxification of malathion. As a consequence, malathion is available to inhibit cholinesterase in the same manner as other phosphates. A parallel mechanism has been shown for EPN action upon the degradation of dimethoate, a carboscyamide (*30*).

After the mechanism of EPN potentiation of malathion became apparent, other phosphate and carbamate pesticides have been examined for their inhibitory effect on aliesterase enzymes (*17–19*). All organophosphorus insecticides inhibit the aliesterases that hydrolyze tributyrin and diethylsuccinate as well as cholinesterases but the marked potentiators of malathion toxicity inhibit aliesterases at a lower dietary level than the level necessary to inhibit cholinesterases (*18*).

As a consequence of these observations, the FDA has modified its regulations on testing for potentiation (CFR 130.35) to "require special experimental data" on a case by case basis as deemed necessary, rather than requiring measurement of the toxicity of each possible combination. *In light of present knowledge, studies on the inhibition of plasma and liver aliesterase should be required on all anticholinesterase pesticides.*

Because all organic phosphate and carbamate insecticides inhibit aliesterases as well as cholinesterase, and because aliesterases are known to metabolize many natural food ingredients, food additives, drugs and industrial chemicals (*20–24*) the public should be protected from exposure to levels of these insecticides that alter the metabolism of other compounds. To define the level for each pesticide that will significantly alter the metabolism of other chemicals, subacute studies have been conducted with rats for most of these insecticides (*18*). When diethylsuccinate and tributyrin were used as substrates, dietary levels as low as 0.5 p.p.m. of some commercial organophosphorus compounds produced 50% inhibition of the activity of these aliesterases. This degree of inhibition significantly reduces the resistance of rats to malathion (*18*). This is important for occupational exposure. However, it is not known whether this degree of aliesterase inhibition significantly alters the rate of metabolism and toxicity of lower doses of malathion or other substrates when the enzyme system is not overloaded. From available evidence (*1, 17*) it appears that it does not, *but more definitive studies should be undertaken. In addition to measuring the effect of aliesterase inhibition on the metabolism of other pesticides, studies should be undertaken to establish the level of aliesterase inhibition that affects the rate of metabolism of ester drugs at therapeutic dosages.*

The role of aliesterases in detoxification reactions in man are poorly defined. The relatively low toxicity of malathion in the rat is dependent on detoxification by aliesterase(s) primarily in the liver. In man the blood aliesterase activity for substrates such as diethylsuccinate (*25, 26*) is extremely low and the more important information concerning levels in the liver has not been obtained. The amidase which degrades dimethoate is less active in human liver than in liver of six other vertebrates (*31*). If the aliesterase activity in the liver of a man parallels blood in being much lower than it is in rats, this may provide an explanation for the higher toxicity of malathion to man than to experimental animals (*9, 27*). Comparative studies on the susceptibility of experimental animals and man to aliesterase inhibition have been conducted with only one organophosphorus insecticide (*26*) and it only involved measurements on the activity of blood of man. Although tributyrinase activity was more strongly inhibited than cholinesterase in the case of the rat, there was no significant difference in susceptibility of the enzymes in the blood of man.

Therefore, from available evidence on one aliesterase in blood, the significance of aliesterase inhibition from organic phosphates to human health would appear to be less important than cholinesterase inhibition except perhaps for occupational exposure. This conclusion, however, is based on less than adequate information on human susceptibility to aliesterase inhibition. *Additional studies should be conducted to evaluate the significance of aliesterase inhibition in man and especially liver aliesterase; and to study the relation between aliesterases and amidases.*

Current total diet studies establish that the level of organophosphorus insecticides and carbamates in the diet of man from agricultural use is below the level which affects cholinesterase and aliesterase activity (*28*). Occupational exposure of applicators and farm workers, however, has frequently resulted in measurable cholinesterase inhibition and presumably aliesterase inhibition (*29*). An increase in the use of organic phosphate and carbamate pesticides will increase the exposure of both agricultural workers and consumers of agricultural products. *This increased health hazard must be monitored carefully* to avoid an increase in susceptibility of the population to toxic actions of other chemicals. *The cumulative effect on cholinesterase and aliesterase activity of wildlife which receives a greater exposure sould also be evaluated* to preserve desirable species.

Although carcinogenic and potential carcinogenic or mutagenic interactions are important, a full consideration of the pharmacological results of interaction in these regards has been left to the respective subcommittees appointed to assist with these topics.

SUMMARY

The most pronounced cases of known interaction associated with pesticides have involved organophosphorus insecticides. The mechanism for these interactions has been demonstrated to be due to inhibition of the detoxifying enzymes referred to as aliesterases. Although all commercial organophosphorus and carbamate insecticides inhibit aliesterase(s) as well as cholinesterases, the marked potentiators inhibit aliesterases at a lower dosage than is required for cholinesterase inhibition. It is prudent to require information on the effect of all anticholinesterase pesticides on aliesterase activity. The evidence on experimental animals indicates that the current level of pesticides in the diet does not inhibit aliesterase(s). However, occupationally exposed individuals may have reduced activity and may be more susceptible to other compounds. Additional work is needed to define the significance of aliesterase activity in man and his relative sensitivity in relation to experimental species.

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Pesticide Interactions Through Alteration of Hepatic Microsomal Enzyme Activity

The second important mechanism by which pesticides are known to produce interactions is by altering the activity of the enzyme systems in hepatic microsomes that catalyze the metabolism of many foreign chemicals. These microsomal enzymes, which are frequently referred to as mixed function oxidases or drug-metabolizing enzymes, are readily induced by many chemical agents and inhibited by a few types of chemical compounds. A pesticide capable of causing induction or inhibition of these enzymes will alter the susceptibility to drugs or other chemicals that are normally metabolized by these enzymes.

During recent years a tremendous amount of attention has been given to induction of hepatic microsomal drug-metabolizing enzymes by various chemical agents. It is well established that elevation of the levels of these enzymes will accelerate the biotransformation of many substrates (pesticides, drugs, and other chemicals) that are normally metabolized by these enzymes. Acceleration of the metabolism usually results in more rapid detoxification but in some cases the metabolites are more toxic than the parent compounds. The extent to which this type of interaction occurs from combinations of pesticides, drugs, and other chemicals has become a question of considerable concern. The number of combinations of pesticides and other chemicals is too great to permit assessment of their potential for interactions by toxicity tests on every possible combination. Thus an understanding of the mechanisms responsible for interactions seems to offer the most practical approach to the development of procedures for measuring the capacity of one agent to alter the toxicity of another substance. This approach is the one that is considered most feasible to detect interactions that may be caused by enzyme induction.

The initial observation that certain foreign compounds may stimulate microsomal enzyme activity was made by Brown, Miller, and Miller (*1*) and extended by Conney, Miller and Miller (*2*) who demonstrated that several carcinogenic hydrocarbons stimulate the synthesis of certain oxidative drug-metabolizing enzymes in liver microsomes. This observation was followed by the finding (*3*) that barbiturates induce microsomal enzyme activity. At the present time more than 200 drugs, carcinogens, pesticides and other chemicals have been reported to cause induction of microsomal enzyme activity. This subject has been extensively reviewed by Conney (*4*) and the classes of chemicals that have been observed to cause enzyme induction at some dose level in one or more species have been tabulated.

The ability of drugs and other chemicals to induce synthesis of microsomal enzymes is of considerable importance in connection with pesticide toxicology since the toxicity of many pesticides would be expected to be altered by changes in the level of drug-metabolizing enzymes in the liver. The possible practical significance of drug-induced increases in microsomal enzyme levels in connection with alterations in pesticide toxicity is difficult to evaluate because many of the observations to date have been made using high doses of the drugs and methodology which is not sufficiently quantitative.

Additional interest in enzyme induction as it relates to pesticide toxicology followed the accidental discovery that chlordane sprayed in animal rooms caused induction of drug-metabolizing enzymes (*5*). This finding had greater significance than many of the observations using high doses of drugs because enzyme induction by chlordane occurred under practical conditions of use thus demonstrating that enzyme induction can occur from environmental contamination. The observation that chlordane causes enzyme induction was soon followed by experiments (*6–11*) which indicated that DDT and other chlorinated hydrocarbons produce this effect. No other class of pesticides has been demonstrated to resemble chlorinated hydrocarbons in potency as enzyme inducing agents.

The organic phosphate insecticides do not cause enzyme induction. Instead some of these compounds have been reported to be inhibitors of microsomal enzymes. In this connection Rosenberg and Coon (*12*) found that organic phosphates prolong hexobarbital sleeping time. Welch *et al.* (*13*) found that several organic phosphates inhibit the metabolism of testosterone possibly by serving as competitive substrates for microsomal enzymes. Recent unpublished experiments (*14*) in which nearly all of the organic phosphates for which tolerances have been established were given daily to mice at one-fifth of the acute LD_{50} for 5 days indicated that a few of these compounds caused moderate inhibition of the activity of two microsomal enzymes but most of them had no effect on drug-metabolizing enzymes. It, therefore, seems reasonable to conclude that the organic phosphates would not inhibit drug metabolism catalyzed by microsomal enzymes at the levels of the organic phosphates that are normally in the environment.

The toxicity of organic phosphates and carbamates is altered by exposure of animals to enzyme inducing agents. In nearly all cases this interaction renders these anticholinesterases less toxic. A recent study of the effects of pretreatment of rats and mice with phenobarbital on the toxicity of 15 organic phosphates showed that the enzyme inducer either had no effect or reduced the toxicity except for one (octamethyl pyrophosphoramide) whose toxicity was increased (*15*). In mice, pentobarbital increased the toxicity of two organophosphates and decreased the toxicity of three organophosphates and two carbamates (*26*). It thus seems likely that exposure to broad spectrum inducers such as phenobarbital or chlorinated hydrocarbons will not often increase the toxicity of organic phosphates. This generalization apparently cannot be extended to the more selective inducers because enzyme induction by 3-methylcholanthrene increases the toxicity of Guthion (*16*) probably because of acceleration of the activation process to a greater extent than detoxification.

The substituted urea herbicides represent another class of pesticides that cause induction of hepatic microsomal enzymes (*17, 18*). However, the no-effect dietary level for enzyme induction by these compounds appears to be between 100 p.p.m. and 250 p.p.m. and their potency as enzyme inducers is, therefore, much less than that of the chlorinated hydrocarbons. Enzyme induction has not yet been observed with most other types of pesticides. Some of the compounds that have been considered for use as chemosterilant insecticides would be expected to decrease the activity of hepatic microsomal enzymes since alkylating agents (*19*) are inhibitors of microsomal enzymes *in vivo* probably through inhibition of protein synthesis.

The number of drugs and other chemical agents that cause enzyme induction is impressive but the percentage of chemicals used for all purposes that produce this effect is unknown because negative results are usually not reported. In a survey of a large number of miscellaneous industrial chemicals, pesticides, and drugs being conducted at the present time (*20*) by repeated parenteral administration of sublethal doses to rats and mice enzyme induction has been a rare occurrence.

The incidence of enzyme induction among pesticides is nevertheless sufficiently high to warrant consideration of this effect in the toxicological evaluation of new pesticides. *It is recommended that tests for enzyme induction be made part of the required studies on all new pesticidal chemicals.* Since many pesticides are used not only on agricultural crops but also in a variety of formulations for household and other uses, the initial tests for enzyme induction could be done using doses higher than those to which exposure would occur under any condition of normal usage. Enzyme induction is a dose-related effect. If no induction occurs with repeated high doses no further consideration would need be given to the possibility of interactions due to enzyme induction at lower doses.

If enzyme induction is observed in the initial tests with high doses of a pesticide, it is important to ascertain dose-response relationships for the effect. *Quantitative measurements of enzyme induction should be made during the subacute or chronic experiments as part of the toxicological evaluation unless separate experiments of a short-term type as described above have shown that the chemical agent is not capable of producing this effect.*

Relatively little attention has been given to measurements of the no-effect dietary level for pesticides that cause induction, although this information is essential for determining whether or not enzyme induction could occur from the permissible and the actual levels in food. This type of study has been done with DDT and toxaphene by measuring the effects of various dietary levels on the activity of three microsomal enzymes (*21*). Dose-related increases in activity of the enzymes was observed and the lowest dietary levels that caused a significant induction of one or more of the enzymes was 1 p.p.m. of DDT and 5 p.p.m. of toxaphene. The theoretical intake of DDT calculated from major U.S. tolerances for the period of 1964 to 1967 was

6.79 mg. per day and the actual intake in the U.S. total diet for that period was 0.037 mg. per day (*22*). Both of these levels are below those needed to produce enzyme induction in rats on an equivalent weight basis.

There is need for quantitative data on enzyme induction by low dietary levels of other chlorinated hydrocarbons. Such measurements are in progress for chlordane, gamma chlordane, heptachlor, endrin, aldrin, dieldrin, methoxychlor, lindane, and heptachlor epoxide fed at various levels in the diet to rats (*23*). Other experiments are in progress which indicate (*24*) that a dietary level of 0.5 p.p.m. of dieldrin increases the metabolism of 6-chloro-17-acetoxy progesterone in dogs.

The extent to which additive induction is obtained by combinations of chlorinated hydrocarbons and by chlorinated hydrocarbons plus drugs is a logical extension of work on individual compounds. Information of this type is needed before a final judgment can be made concerning the significance of the total intake of enzyme inducers from the standpoint of interactions.

In view of the large number of different types of chemicals that are used as pesticides or that might be developed for that purpose in the future, *more research aimed at finding ways to predict enzyme induction on a structure-activity basis is needed.*

Species differences in response must be considered in the extrapolation of enzyme induction data obtained on experimental animals to man. *More research is needed to establish quantitative and qualitative criteria to indicate similarities and differences in drug metabolism in various species. The extent to which the low levels of microsomal enzyme activity in extrahepatic tissues such as lung, gastrointestinal tract, kidney, and adrenal cortex is induced by pesticides should be studied.*

Accomplishment of much of the needed research is dependent upon appropriate methodology. Methodology applicable to human studies is especially needed. In general, enzyme induction can be demonstrated by measuring (a) levels of microsomal enzymes in liver preparations, (b) duration of action of drugs known to be metabolized by microsomal enzymes such as hexobarbital, phenylbutazone and antipyrine, (c) urinary excretion of natural substrates such as ascorbic acid and 6-b-hydroxycortisol, and (d) changes in liver weight and hepatic endoplasmic reticulum. All of these measurements are concerned with the end-result of a biochemical process which is not yet understood at the molecular level. Thus there are probably more limitations to their predictive value than would be the case if direct measurement could be made of the reactions affected. For example, some organic compounds containing trifluoromethyl groups selectively induce O-demethylase activity without causing detectable changes in liver weight and without affecting the metabolism of drugs by other microsomal enzymes (*25*). *More research is needed on the development of practical quantitative methodology for measuring enzyme induction in animals and man. Consideration should be given to the development of model substrates with low toxicity whose metabolic products can be easily measured.* Substrates of this type would be extremely useful for measuring enzyme induction in man by following the rate of appearance of metabolites in the blood. They would also be useful in chronic animal toxicity tests where periodic tests for enzyme induction seem desirable in view of the tendency of some chemicals to produce an initial marked induction followed by a return toward normal levels (*21*). A worthwhile goal would be the development of methodology for measuring enzyme induction that could be applied as readily as the standard liver function tests. When a suitable method is available for determining the level of enzyme induction in man, *monitoring studies should be conducted on individuals exposed in the manufacture and application of pesticides.* Whereas dietary levels of chlorinated hydrocarbons do not appear to be high enough to induce microsomal enzymes, there is insufficient quantitative data to form a conclusion regarding induction from occupational exposure.

SUMMARY

The ability of some pesticides, especially the chlorinated hydrocarbons, to induce hepatic microsomal drug-metabolizing enzymes has been well established in both experimental animals and human beings. Thus, there is a possibility that practical levels of these pesticides could cause interactions by altering the rate of metabolism of drugs, other pesticides, and industrial and environmental chemicals. However, where quantitative data are available for the dose-response relationships for enzyme induction and for the dietary intake of pesticides, it is concluded that the present intake is insufficient to cause interactions resulting from enzyme induction. For DDT sufficient information is available to permit this conclusion with reasonable certainty, unless man responds to a greater extent than experimental animals.

Less is known about the effects of occupational exposure to pesticides on microsomal enzyme levels and it is possible that some of these exposures may cause an alteration in the metabolic rates of drugs and other chemicals. More attention should be given to enzyme induction as new pesticides are developed and there is a great need to quantitate the enzyme inducing effects so that their significance at practical dose levels can be estimated. Additional research is needed on methodology for measuring enzyme induction in various species and especially in man.

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Pesticide Interactions at the Target Level

As indicated in the intoduction to this report, the Committee focused attention on mechasims of established importance as a cause of pesticide interactions. It was concluded that interactions at the target or receptor level were among those for which there is no documented evidence. However, there have been two claims that interactions involving insecticides result from events occurring at the central level. It was, therefore, considered necessary to present the reasons for the opinion that interactions at the target level have not been adequately demonstrated.

In 1962 Karczmar (*1*) reported that when EPN was given *after* malathion synergism still occurred and he doubted whether under these delayed conditions the effect could be attributed to blockade by EPN of malathion metabolism. However, this opinion ignored the possibility that there was, at the time of administration of EPN, enough malathion left to produce a toxic effect in which case blockade of its degradation would be expected to give rise to synergism. Later Karczmar *et al.* (*2*) showed that in dogs and cats, EPN and malathion were synergistic with respect to promptly elicited effects on the neuromuscular junction and the brain (as judged by their own electroencephalogram recordings). They postulated either a joint effect upon the receptor or a "sensitization" of cholinesterase by one of the compounds. Although the purity of the sample of malathion used in these studies was not stated, the *in vitro* anticholinesterase activity reported for the compound ($8 \times 10^{-6}M$) indicates that it was extremely impure. The interpretations placed on these findings by Karczmar have not been accepted by others (*3*). It seems especially unlikely that for this pair of compounds, for which such ample evidence exists that the interaction is due to EPN blockade of degradation, effects occur at the level of the receptor.

It is extremely difficult to imagine a mechanism by which two inhibitors acting upon a single target, whether it is cholinesterase or a receptor, can synergize each other. As in the strictly parallel case of the action of one enzyme upon two substrates, the interactions can only be additive or antagonistic. However, the possibility of synergism could arise with two compounds whose final effects are upon a single system yet mediated through two different mechanisms. One plausible possibility would be an axonic agent (for instance a chlorinated hydrocarbon) which gave rise to hyper-excitability of the axon and therefore enhanced release of transmitter substance such as acetylcholine at the synapse; coupled with an anticholinesterase agent which impaired the ability for acetylcholine destruction at the synapse. The possibility of such an interaction has not been explored in any detail, and it certainly deserves a modest research effort. The only relevant observation in the literature is that of Ball *et al.* (*4*) and Crevier *et al.* (*5*) who found a contrary effect. After feeding aldrin, chlordane, or lindane there was marked protection of rats against subsequent parathion poisoning. It is now believed (*6*) that this effect was due to stimulation of the liver microsomal enzymes by the chlorinated hydrocarbons. Consequently, such experiments do not provide the ideal context in which one might observe synergism at the target level.

SUMMARY

No cases of interaction at the target level have ever been satisfactorily demonstrated. The possibility of such interaction may always exist, but until demonstrated it cannot be a significant factor in evaluation of health hazards. No special search was made for carcinogenic or mutagenic effects of interaction at the target level.

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Relation of Interactions to Tissue Storage of Persistent Pesticides

The storage and elimination of pesticides from tissues is a process that can be affected by the ability of pesticides or other chemicals to stimulate or inhibit the detoxification processes discussed in the first two sections of this report. For this reason it was considered important to include information on the effects of interactions on the storage of pesticides in tissues.

In recent years a considerable amount of information has been obtained regarding the occurrence in human tissues of small residues of several of the more persistent pesticides, particularly those comprising the organochlorine insecticides and their several metabolic products. Among the most important materials in this group are p,p'-DDT, p,p'-DDD, p,p'-DDE, o,p'-DDT, the isomers of BHC, and the cyclodienes, dieldrin and heptachlor epoxide. Of the total chlorinated organic residues found in consumer products during the years 1966–68, DDT and its analogs constituted approximately 67 percent, while lindane (γ-BHC), dieldrin and heptachlor epoxide combined, contributed an additional 12 percent (1). This section of the report will, therefore, be concerned largely with these particular pesticides. Appendix A reviews the status of information about tissue levels of these pesticides as a function of intake. The occurrence of significant tissue residues of most other groups of commercial pesticides such as the carbamates and organophosphates is in general obviated by their rapid biodegradation and subsequent elimination from the body. These compounds will, therefore, not be given further consideration.

Effects of interaction on pesticide storage. Much of the present data on storage of the organochlorine pesticides has been obtained from controlled laboratory experiments in which animals or human volunteers have been exposed to known amounts of a single pesticide. Although the results of such investigations are often extremely useful, the fact that the population is presently subject to simultaneous exposure to a combination of many drugs, pesticides, air pollutants, food additives and cosmetics, raises questions regarding the possibility of interactions between these various synthetic chemicals. Furthermore, in assessing potential hazards it is important to consider the possibility of storage interaction in those members of the population under various types of physiological stress, such as those resulting from weight loss or surgical procedures.

Interactions could affect either the rate of accumulation of pesticides in the tissues, the steady state level, or the rate at which tisue depletion occurs following termination of exposure. It should be made clear from the outset that very little data are available on interactions involving storage.

The concentration of organochlorine pesticides in human tissue results from a dynamic equilibrium involving on one hand the rate and level of intake, and on the other, the rate of metabolism and/or elimination. Consequently, interactions which either increase the effective intake or which decrease the rate of metabolism could conceivably increase the steady state levels in the tissues.

The intake of a pesticide can be modified for example by the type of formulation in which it is dispersed, and interaction with various spray additives such as emulsifiers, surface active agents and organic solvents could be particularly significant in determining tissue accumulations in the occupationally exposed. It is also possible that interactions of pesticide residues with detergents used in the home could lead to enhanced absorption of ingested materials from the alimentary tract by the general population and indeed surface active agents have been found to increase the oral toxicity of DDT and other insecticides to the rat (*2*). The nonionic wetting agent "Tween 20" has been shown to increase tissue levels of the β-isomer of BHC in rats fed on a diet containing relatively high levels of a combination of these materials (*3, 4*). It was also shown that the level of storage increased with the percentage of fat in the diet and it was concluded that the increased storage of γ-BHC was due to an enhanced gastrointestinal absorption. At trace residue levels, it seems probable that efficient absorption takes place and dietary variation would not significantly increase absorption or storage. However, no direct evidence is available to support this conclusion.

Many household pesticide formulations contain insecticide synergists of the methylenedioxyphenyl type which are added to enhance the insecticidal action of the pyrethrum insecticides. As discussed elsewhere in this report synergists of this type are able to inhibit the hepatic microsomal enzymes that are largely responsible for insecticide detoxification. Consequently, in the case of a material which must undergo microsomal modification before excretion from the body the presence of a synergist residue could conceivably prevent the elimination and result in enhanced tissue levels. The currently limited commercial use of insecticide synergists (household formulations) and probably insignificant residue levels make this type of interaction unlikely at the present time. *However, in the event of the possible widespread application of insecticide synergists to agricultural crops at some future time and the possibility of the development of more stable synergists, such interactions should be considered. Information should then be obtained on the effect of insecticide synergists on tissue levels of persistent pesticides.*

A considerable amount of work has been carried out in recent years on the storage interactions of combinations of organochlorine insecticides, particularly dieldrin and DDT. Simultaneous feeding of rats with dieldrin and DDT was observed to have a marked effect in decreasing the levels of dieldrin storage in the tissues (*5*, *6*, *7*) and this has subsequently been found to occur with all of the cyclodienes investigated. Similar but less marked effects have also been observed with DDE, DDD, and DDMU. In combination with 50 p.p.m. of DDT the levels of dieldrin and heptachlor (fed at 1 p.p.m. in the diet) in rat adipose tissue were reduced by factors of 15 and 11 respectively as compared with the controls. Significant reduction of dieldrin storage was also observed with DDT at 5 p.p.m. A greater relative response in terms of dieldrin storage results from both low dieldrin dosages and higher levels of DDT. It is suggested that significant interaction might be expected to occur at dietary levels of 0.5 to 1.0 p.p.m. of DDT and 0.1 p.p.m. of dieldrin (*7*). Although these levels are considerably greater than the present total daily intake figures for the general population (total diet studies) they are of the same order as the estimated tolerance levels established by the FDA, and could certainly fall within the levels resulting from occupational exposure.

It has been suggested that the mechanism through which these storage interactions occur might involve microsomal enzyme induction (*7*) which would result in a stimulation of metabolism and a consequent enhancement of the rate of elimination of pesticides from the body. Many of the chlorinated hydrocarbons are potent inducers of liver microsomal enzymes (*8*, *9*) and DDT is reported to be one of the most potent in this respect (*10*). Certainly the depletion of dieldrin from the tissues following administration of DDT is associated with an enhanced excretion of hydrophilic products of dieldrin metabolism in both the urine and feces (*7*, *11*). The effect of DDT on dieldrin storage was observed to become maximal after about 3 days and was maintained at this level for up to 10 weeks with continued administration of DDT, thus indicating the establishment of a new steady state level in the tissues. Following the termination of exposure to DDT the effect on dieldrin tissue levels persisted for more than 6 weeks (*7*).

In addition to its effects on the storage levels of dieldrin and other cyclodienes, DDT has been shown to change the metabolism of lindane (*7*) and of DDT itself (*12*) and it is, therefore, probable that metabolic interactions of this type could play a significant role in determining or modifying the steady state storage levels of these materials.

Furthermore, it has been shown that a number of drugs including phenobarbital (*13*), aminopyrine, phenylbutazone, tolbutamide and chlorpromazine (*7*) all of which can induce microsomal enzymes, are similarly able to significantly reduce the observed levels of dieldrin storage. The one major question which challenges the suggestion that microsomal enzyme induction by DDT is the sole cause of accelerated dieldrin depletion is the repeated failure of inhibitors of protein synthesis to inhibit the process (*7*). Both ethionine and actinomycin D are inhibitors of protein synthesis and have been found to inhibit DDT induction of microsomal enzymes (*14*).

Much of the currently available data on insecticide interactions and microsomal enzyme induction have been obtained from investigations with laboratory rodents. That species differences may exist, particularly with regard to storage interactions, should not be overlooked. This is emphasized by the results recently reported by Deichmann *et al.* (*15*) who investigated storage interactions in dogs fed on diets containing aldrin and DDT either alone or in combination. These workers found that tissue levels of dieldrin remained essentially the same in dogs fed for 10 months on a diet containing either 0.6 mg./kg. of aldrin or a combination of 0.3 mg./kg. of aldrin and 12 mg./kg. of aldrin and 12 mg./kg. of DDT. These results are in marked contrast to those obtained by Street (*7*) using rats. Deichman *et al.* (*15*) also investigated the effect of aldrin on the tissue retention of DDT. Dogs fed for a period of 10 months on a diet containing 24 mg./kg. of DDT had tissue levels of this material of 547 p.p.m. Dogs fed on a diet containing 12 mg./kg. of DDT plus 0.3 mg./kg. of aldrin, however, had a considerably higher tissue concentration of 1,290 p.p.m. of DDT after a similar 10-month period. Although at a much lower level, DDE in the tissues of dogs showed a similar pattern. The results of preliminary investigations on the concentration of DDE in the blood of human volunteers fed for 2 years with 211 μg. of dieldrin per day have shown no significant decrease in DDE levels during this time (*16*). This is not entirely unexpected, however, since it is unlikely that DDE levels would be affected by microsomal enzyme induction. Neither the formation of DDE from DDT nor the subsequent metabolism of DDE itself are known to be carried out by microsomal enzymes. The relevance of these contrasting data to man is not known, and more research should be carried out in this area.

Some possible beneficial effects from enzyme induction have recently been recognized and are currently being investigated by R. M. Cook and colleagues at Michigan State University. Using nonpersistent drugs such as phenobarbital, it has been found possible to flush out of animals relatively high concentrations of persistent organochlorine pesticides such as dieldrin. A successful application of this technique has recently been reported (*17*) in which a dairy herd was accidentally exposed to aldrin-contaminated feed. Treatment of the cows with 2 lbs. of activated charcoal per day plus 5 g. of phenobarbital resulted in a marked decrease of dieldrin in only 39 days. Other experiments along these lines are currently in progress (*18*).

There is also recent evidence that inducers such as phenobarbital may have considerable therapeutic potential for decreasing the levels of chlorinated hydrocarbons in humans, and may be particularly useful in cases of accidental poisonings. A report by Davies *et al.* (*19*) indicates that patients taking the anticonvulsant drugs phenobarbital and phenytoin (diphenylhydantoin) for periods longer than 3 months had strikingly lower blood levels of DDE than the general population. Healthy controls, not taking the drugs had a mean concentration of 9.1 p.p.b. of DDE in the blood, compared with levels of 3.5 and 1.9 p.p.b. respectively in outpatients taking phenobarbital and phenytoin alone and 1.7 for those patients taking both drugs. Levels of DDT-derived materials in the adipose tissues of severely retarded, nonambulant inpatients not taking drugs indicated a mean value of 2.70 p.p.m., whereas similar patients undergoing regular anticonvulsant therapy with one or both of the above drugs had a mean tissue level of only 0.17 p.p.m. The mechanism of this effect is suggested to be enzyme induction, although as previously suggested it seems unlikely that DDE is metabolized by the microsomal enzymes. The fact that phenobarbital is a known antidote for acute DDT poisoning is interesting in that it may have a dual effect, acting not only to suppress the acute central symptomology of DDT action, but also aiding the body in eliminating the material.

Physiological interactions.—It has been suggested (*20*) that toxic symptoms could result from an increase in dieldrin in the body tissues following weight loss through either sickness, dieting, or increased catabolism in response to injury. This phenomenon was first demonstrated by Fitzhugh and Nelson (*21*) in rats starved completely after being fed diets containing 600 p.p.m. of DDT. Subsequent investigation (*22*) showed that partial starvation of rats fed DDT at a rate of 200 p.p.m. in the diet led to increased levels of DDT-derived material in all tissues resulting from the mobilization of body fat. The above studies were, of course, carried out with intakes of DDT several orders of magnitude greater than those encountered in pesticide residues.

Recent evidence (*16*, *23*, *24*) indicates that neither surgical stress nor complete starvation causes any significant changes in the levels of dieldrin in human blood in spite of considerable losses in the body depot fat.

In the case of the lipophilic organochlorine compounds it is probable that absorption of the materials from the intestine can be increased in combination with high fat diets. Frawley (*3*, *4*) showed that storage of γ-BHC by rats fed on a diet of 8-percent fat was consistently 2 to 3 times that in rats fed on a 5 percent fat diet.

The effects of other nutritional factors on the storage levels of pesticides in body tissues have received little attention. Storage of dieldrin in the tissues of vitamin A-deficient rats has been found to be almost double the storage in controls (7) and Street suggests that this may result from an impairment of microsomal enzyme activity.

SUMMARY

Small residues of the persistent organochlorine pesticides and their metabolites are found in the body tissues of humans. The actual levels occurring reflect a dynamic equilibrium between the degree (level and duration) of exposure and the rate of elimination from the body. In the general population it appears that a steady state equilibrium has been established and this is likely to be maintained under current patterns of pesticide usage. These low levels of tissue residues are unlikely to present any hazard to the general population. In view of the large number of chemicals to which man is currently exposed, the occurrence of pesticide-pesticide, pesticide-drug, or pesticide-"*x*" interactions with regard to tissue storage are possible, but from the information currently available this does not seem to constitute any cause for concern. Storage levels of the organichlorine pesticides could be increased in combination with residues of detergents through facilitated absorption from the gut, and residues of insecticide synergists could result in increased storage levels as a result of inhibition of the microsomal enzymes. There is a greater probability that interactions could lead to a depletion of tissue storage levels through the induction of microsomal enzyme activity and consequent enhancement of pesticide elimination. The use of short lived drugs for inducing liver microsomal enzymes has therapeutic potential for clearing persistent residues from animal and human tissues.

If the use of insecticide synergists increases, information should be obtained on the effect, if any, on storage levels of organochlorine pesticides. Information should be obtained on microsomal enzyme induction in man and its effect, if any, on storage levels of persistent pesticides in human tissues. Further work should be encouraged on the effect of storage by combinations of pesticides with household detergents.

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Exogenous Physical Factors Influencing Pesticide Toxicity

Most of the available information on pesticide interactions concerns the ability of one chemical agent to modify the toxicity of another one by the mechanisms discussed in previous sections of this report. However, there is growing recognition that physical environmental factors such as temperature or even social factors (crowding) can also modify responses to pesticides. Whether such modifications constitute an example of pesticide-physical factor interaction in the same sense as occurs with pesticide-chemical factor interactions depends in part on the magnitude of the effect and in part on how interaction is defined (potentiation versus additive effects, reciprocal versus one-way actions, etc.). It is evident, however, that in evaluating the hazards of pesticide exposure, consideration must be given to the possibility that these hazards can be influenced by variation in the physical as well as the chemical factors of the environment. For this reason the present status of this aspect of pesticide toxicology was considered worthy of inclusion in this report.

The two physical factors in our environment that have been shown to be capable of altering the response to subsequent pesticide exposure are temperature and radiation. Diet is another exogenous factor that has been included in this discussion because of the possibility that its physical characteristics may influence pesticide response. However, the majority of its known effects are related to chemical properties such as alteration of the nutritional status of the subject, or the presence of food additives or other chemicals.

Diet. The feeding of low protein diets to rats has been reported to increase susceptibility to the acute toxic effects of captan, lindane, dieldrin, and chlordane but not to DDT (*1–5*). Similar effects have been observed with the anticholinesterase insecticides banol and parathion (*4*). These effects were of sufficient magnitude in the studies with captan (where there was a 25-fold decrease in the acute oral LD_{50}) to cause Boyd and Krijnen to suggest (*1*) caution in the use of this agent in countries where the diet is low in protein. Dieldrin and DDT appear to accentuate the effects of a diet deficient in the essential fatty acids in the rat (*6, 7*) whereas an increase in the fat content of the diet increased the storage and the acute toxicity of benzene hexachloride (*8*) and altered the erythrocyte cholinesterase inhibiting ability of parathion (*9*). On the other hand, dieldrin partially protects rats against the effects of thiamine deficiency but does not protect against the deficiency symptoms of riboflavin and pyridoxine (*10, 11*).

Possible mechanisms for the effect of these dietary variations on insecticide toxicity include a reduced formation of the detoxifying enzymes as a direct result of the low protein diet or a pesticide-induced stimulation of the enzymes that desaturate fatty acids in the low fat diet situation. Recent studies by Kato (*12*) have shown that sex differences which exist in the drug-metabolizing processes of rats fed high protein diets are reduced when the animals are fed low protein diets. A more general mechanism for the effects of dietary variation on pesticide response is that the dietary restrictions and/or the pesticide exposure represent stress situations and the resultant interactions are due to steroid-mediated effects on the microsomal enzyme systems. The enhanced toxicity of benzene hexachloride in the rats fed a high fat diet can be attributed to increased intestinal absorption and this effect can be increased further by the addition of a synthetic emulsifier to the diet (*13*).

Temperature. Rats fed malathion in the diet have been reported to have a reduced ability to withstand the effects of exposure to a cold environment (*14*) whereas a warm environment was observed to increase the toxicity of parathion in rats (*15*) and sarin in monkeys (*16*). Since the organophosphate insecticides as well as other cholinesterase inhibitors have been shown to cause a temporary collapse of thermo-regulation in animals (*17–19*), some types of demonstrable interaction between pesticide exposure and both heat and cold exposure might be anticipated. Similar studies do not appear to have been carrried out with the chlorinated hydrocarbon insecticides or other pesticides; however, diethyldithiocarbamate, pentachlorphenol, and related pesticides are known to influence body temperature in rodents and in man (*20–21*). It is difficult to predict the net result of a combined exposure to pesticides and either heat or cold since body temperature also affects the metabolism, distribution (including protein-binding), and excretion of a variety of endogenous chemicals including pesticides. Recent studies by Murphy *et al.* (*22–24*) have shown that exposure to organophosphate insecticides, chemical irritants, and cold stress produce an increase in the activity of the plasma glucocorticosteroid level with a resultant increase in the activity of the glucocorticoid-inducible rat liver enzymes (tyrosine transaminase, alkaline phosphatase, etc.). Furthermore, several of the organophosphate insecticides have been shown to be capable of inhibiting the microsomal hydroxylation of testosterone in rats (*25*) and at least one of these, chlorthion, inhibits the hepatic metabolism of desoxycorticosterone to its polar metabolite.

Radiation. DuBois *et al.* (*26–28*) have shown that the exposure of young male rats to sublethal doses of whole-body x-irradiation produces a dose-dependent inhibition of the development of the microsomal enzyme system(s) responsible for the desulfuration of certain organophosphate insecticides. Exposure of adult rats to the same doses of radiation had no effect on microsomal enzyme activity. In further studies (*29*), this was shown to be an abscopal effect which could be prevented by shielding of the head during the radiation exposure, and it has been suggested (*30*) that the effect is due to an impairment of the normal hypophyseal regulation. The organophosphate insecticides do not protect animals against the toxic effects of whole-body x-irradiation and such exposure does not markedly alter either the toxic effects of these insecticides or the ability of atropine to antidote the effects of poisoning by these agents (*31*).

Other physical factors. Microsomal drug metabolism appears to be inhibited by hypobaric hypoxia and to be stimulated by hyperbaric oxygen exposure (*32*, *33*). It is likely, therefore, that these environmental situations will also influence the metabolism of pesticides and, perhaps, their ability to induce liver microsomal enzymes. Respiratory depression is a prominent feature of organophosphate insecticide poisoning, and hyperbaric oxygen exposure could exert a beneficial effect on the outcome of such an exposure. Other factors such as humidity, forced exercise, aggregation, restraint, etc., influence both the toxic and therapeutic response to drugs and will probably also do so with pesticides and other chemicals.

SUMMARY

Environmental physical factors are capable of influencing pesticide response in animals and probably also in man. There is, at present, no indication that the physical factors exert a greater effect on pesticide response than the chemical factors in our environment or that these physical effects constitute a hazardous situation for the general public.

Additional research designed to characterize the mechanism(s) and the significance of pesticide interactions should include the study of the effects of the total environment (chemical, physical, and social factors).

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APPENDIX A

TISSUE STORAGE OF PESTICIDES

As a result of the statistical analysis of data, obtained through tissue studies of members of the general population combined with those from selected segments of the population and experimental human volunteers, it is now possible to make certain generalizations regarding the pharmacodynamics of organochlorine insecticides based on a compartmental model (*2, 18, 19*).

It is now clear that the amount of material entering the body, the amount stored in different tissues, and the amount which is metabolized and excreted from the body exists as a dynamic equilibrium. The major storage reservoir is the neutral fat comprising the adipose tissue and this undoubtedly reflects a physiocochemical partitioning of the nonpolar organochlorine materials into these tissues. Thus, although residues can be detected in the tissues of the other organs these are usually markedly lower than the levels found in adipose tissue. Residue levels of chlorinated hydrocarbon insecticides in the liver of humans, for example, are found to be approximately 10-fold lower than those in adipose tissue while levels in kidney, brain, gonads, and blood are smaller by a factor of greater than 100 (*1*). The concentrations of an organochlorine pesticide in different tissues appear to be related to to the function of the organ as well as to its fat content (*2*), and good correlations have been observed between dieldrin levels in adipose tissue and whole blood (*3, 4*), with the concentration ratio being 156 : 1. The measurement of dieldrin in whole blood, therefore, reportedly constitutes a convenient method of assessing the total body burden of dieldrin.

Factors determining storage.—One of the major factors which determines the concentration of pesticide residues in human body tissues is the level of human exposure which directly determines the average daily intake. Thus, the storage of DDT in human adipose tissues has been correlated directly with the exposure (mean daily intake) over a dosage range of approximately 1,000 (*5*) and similarly good correlations have been obtained from dieldrin levels in both whole blood and adipose tissue (*2, 4, 6*).

Clearly, considerable variation exists in the exposure levels of different segments of the population. For the general population the major source of continuous exposure results from residues in ingested food materials. Reasonable estimates of the ingestion of pesticides in food are provided by the total diet studies obtained by the Food and Drug Administration (*7*). Such studies indicate that during the years 1965–68 the average daily intake of DDT is approximately 0.03 mg., which is equivalent to a dosage of about 0.0004 mg./kg./day for an average 70 kg. man, and results in storage levels in the adipose tissue of about 4 p.p.m. (*8*). Similar studies with dieldrin establish an average total daily intake (total diet) of about 0.005 mg. and corresponding adipose storage levels of 0.20 to 0.25 p.p.m. (*8, 2*).

Intake and storage of other organochlorine pesticides are considerably smaller and with the exception of dieldrin all are much lower than the acceptable daily intake (ADI) figures established by the FAO/WHO (*9*). The total daily intake of dieldrin is approximately equal to the ADI figure, but is only about 2 percent of the estimated tolerance intake established by the FDA and based on tolerance levels of pesticides in foods (*10*). Actual daily intake of DDT and lindane amount to only 0.5 and 0.4 percent, respectively, of the theoretical intake calculated from U.S. tolerances. Because of large variations in residue levels associatedwith different consumer products, intake and storage levels of the organochlorine pesticides varies to some extent with food preferences of the individual. Members of the population who consume large quantities of meat, fish, poultry, and dairy products, which when combined, account for over 50 percent of the total daily pesticide intake, can be expected to have higher tissue levels of these materials than meat abstainers (*8, 10, 11*).

Radomski and Deichmann (*1*) have recently established that considerable variation in tissue residue levels within the general population can result from household use of pesticides. Possibly as a result of the prevalence of DDT in household pesticide formulations, residues of DDT and DDE in the adipose tissues of individuals accustomed to heavy use of pesticides around the home were three and four times, respectively, those found in people who used little or no household pesticides. A slight increase was also observed in the case of BHC although no significant differences were found with respect to the cyclodienes, dieldrin, and heptachlor epoxide which are not commonly employed in household formulations. It is clear that this type of exposure may be more important than has been previously suspected.

In addition to the environmental exposure associated with household use of pesticides, it is clear that populations living in areas where regular agricultural spray programs are effected are subject to further atmospheric exposures. Small but significant additional residues of DDT and DDE have been found in the tissues of persons with this type of environmental exposure (*11*, *12*).

Not unexpectedly, highest tissue residues of organochlorine materials are found in those members of the population whose occupations bring them into regular and sustained contact with relatively high concentrations of pesticides. These include among others, spray formulators, applicators, and employees in pesticide manufacturing plants. Blood levels of dieldrin in samples of whole blood from workmen handling aldrin or dieldrin have been found to be up to 80 times those of the general population (*4*, *5*, *13*), and levels of DDT and DDE in the adipose tissues of workers exposed to DDT are approximately three- to four-fold those found in the normal population (*11*, *12*). It is of interest and possible significance that the levels of DDT and DDE in the tissues of the occupationally exposed are approximately the same as that in nonoccupational exposure resulting from household usage (*1*).

Another important factor which determines the accumulation of pesticide residues in body tissues is the time of exposure to the material (*2*, *4*). The results of experiments in which human volunteers were given known daily doses of dieldrin support the view that levels of this material, measured in the blood, increase in a curvilinear manner and show an asymptotic approach to an upper limit (*3*, *4*). This indicates the establishment of a steady equilibrium, the level of which is determined by the average daily intake (*2*). The validity of the existence of such a steady state is further substantiated by the levels of organochlorine insecticides found in tissues of the general population over a period of years. Following the introduction of DDT in about 1941 the storage levels of DDT and DDE in human tissues showed a steady increase until 1950–55 (*10*). Since this time, storage levels have remained more or less constant and there is even some indication of a slight decline during the last few years. Quaife, Winbush and Fitzhugh (*14*) although contesting the evidence of a steady state for DDT between 1950 and 1958 (*11*) concur that it may have existed since that time. Certainly no significant increases have been observed in tissue levels of DDT or dieldrin in samples collected in the Chicago area between 1962 and 1966 (*15*), and storage of dieldrin by the general population of the United Kingdom has not changed significantly since 1961 (*6*). These data are in good agreement with the relatively constant levels found for the dietary intake of pesticides in the United States from 1965 to 1968. (*8*). From available information it can, therefore, be reasonably concluded that storage of the organochlorine pesticides has attained a steady-state level in tissues of the general population, and under present pesticide usage patterns further significant increases are unlikely.

An important fact which arises from considerations of the pharmacodynamics of organochlorine pesticides is that when exposure to the pesticide is terminated, residue levels in the various body tissues are slowly depleted through metabolic and/or excretory processes. The depletion of dieldrin in the adipose tissues of rats placed on a normal diet after being fed for a period of 6 months on a diet containing 10 p.p.m. of this material was found to follow approximately first order kinetics (*2*). Dieldrin levels in adipose tissue fell from approximately 13 p.p.m. to 0.07 p.p.m. in the 80 days following termination of dietary exposure. Although measured at considerably higher levels, a similar decline in DDT-type residues has also been observed to occur in adipose tissues of beef steers treated experimentally with technical DDT (*16*), and to a lesser extent in dogs treated with either DDT alone, or combinations of DDT and aldrin (*17*).

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PESTICIDE INTERACTIONS WITH SYNERGISTS

Chemical agents used as insectide synergists are of considerable importance in connection with the subject of interactions since the basis of their usefulness depends upon their ability to increase the toxicity of insecticides to insects. Mechanisms similar to those involved in producing increased toxicity to insects might also be operative to alter detoxification systems or other reactions in man. Thus the possible ability of insecticide synergists to produce interactions should be given careful attention in evaluating their safety.

At the present time the methylenedioxyphenyl compounds, of which piperonyl butoxide is a member, are the outstanding important examples of insecticide synergists. These agents at high concentration act as inhibitors of hepatic microsomal enzymes *in vitro* and *in vivo*. As such, they represent only one of the many types of compounds that have the ability to alter the metabolism of drugs, pesticides, and other chemicals. The implications of this type of action have been discussed in previous sections of this report. However, because of the potential importance of insecticide synergists and the need for greater dissemination of information about their toxicity, actions, and metabolism, a rather detailed account of the toxicology of these compounds, which goes beyond the scope of the immediate subject of this report, has been prepared. This information is included below as appendix B.

APPENDIX B:

TOXICOLOGY OF SYNERGISTS FOR PYRETHROID INSECTICIDE CHEMICALS

SUMMARY

Methylenedioxyphenyl synergists, such as piperonyl butoxide, which are used to enhance the insecticidal activity of pyrethroids, are effective inhibitors, both *in vitro* and *in vivo*, of liver microsomal drug metabolism. These compounds are also inducers of liver mixed-function oxidase activity. Their potency in altering drug metabolism in mammals, by either inhibition or induction, is not unique and frequently is lower than that of other insecticidal and pharmaceutical chemicals. Dramatic interactions with other toxicants or biologically active materials can easily be demonstrated by treating mice or rats with high doses of piperonyl butoxide or related compounds; however, these doses are extremely high relative to normal use and exposure conditions. The MDP synergists are very useful in insect control, function by a mode of action that is relatively well understood, and are readily metabolized following oral administration. Although current evidence does not indicate that the use of piperonyl butoxide is a hazard to health, further investigation is needed to evaluate its effect and metabolism. The possible interaction of piperonyl butoxide when inhaled or ingested with a variety of other toxic substances such as pesticides or environmental chemicals or air pollutants, also requires further study. In development of additional synergists, and particularly those of high potency and increased biological or environmental stability, tests on mixed-function oxidase inhibition should be included in the safety evaluation.

A synergist is generally used with pyrethrum to minimize the amount of insecticide necessary for insect control, thus making it economically feasible to use this expensive natural product for control of household pests. The same situation applies to the synthetic pyrethroids. Certain synergists also enhance the toxicity of several other insecticide chemicals, including rotenone, carbamates, and certain organophosphates and chlorinated hydrocarbons, although they are not being used in many areas of insect control, because economic benefits are not realized from such use. The information available on the nature of synergism of these compounds is summarized in reviews by Metcalf (1955, 1967) and Hewlett (1960, 1968).

The most important commercial synergist employed in insect control is piperonyl butoxide, a methylenedioxphenyl (MDP) compound prepared from dihydrosafrole in an amount of about 800,000 lb per year.

Other important commercial or experimental MDP synergists are sulfoxide, prepared from isosafrole, and tropital, prepared from piperonal. The use of these materials is limited not only by toxicological and economic considerations but also by the fact that they are all prepared from a natural product, safrole, which is available in the amount of only about 1,400,000 lb per year (Hennessy, 1969).

There are also other compounds active as synergists for pyrethroids, including MGK-264 which is used commercially and certain propynyl phosphorus compounds and aryl propynyl ethers which are under consideration for development. The remarkable synergism noted for the insecticidal activity of many carbamates, particularly carbaryl, with MDP and other synergists has not proven economically beneficial under actual field-use conditions. It is possible that, properly used, synergists could help to (1) reduce the necessary dose of toxicant and therefore of environmental contamination, (2) minimize the degree of resistance in tolerant insect species or strains, and (3) permit the use of biologically unstable, biodegradable, or nonpersistent compounds by blocking detoxication mechanisms for these toxicants in the pest. Although there is reason to believe that the use of synergists will increase in the future, the area of immediate concern is the potential hazard, if any, from the use of MDP compounds, and particularly of piperonyl butoxide.

MAMMALIAN TOXICOLOGY OF METHYLENEDIOXYPHENYL COMPOUNDS

Acute, subacute, and chronic oral toxicity and pathological studies with piperonyl butoxide using different animal species were conducted by Sarles and Vandergriff (1952). Piperonyl butoxide was found to be of relatively low toxicity to rats, dogs, goats, and monkeys. There was little difference between sex and species in susceptibility to this material, and no indication was observed of a cumulative toxic effect upon the second and third generation of rats fed a diet containing up to 10,000 p.p.m. of piperonyl butoxide. The outstanding biological effects of ingestion of relatively large doses of piperonyl butoxide were anoxia, wasting, reduced food consumption, reduced or lost ability to reproduce, and accentuation of concomitant natural disease. Although piperonyl butoxide was not carcinogenic for the liver, nor did it exert a malignant tumorgenic effect upon the general tissues, endocrine glands, or breast, it did produce nonspecific lesions in the livers of the test animals. A safe human tolerance for chronic ingestion of piperonyl butoxide was estimated, after allowing a 100-fold safety factor, to be 42 p.p.m. in all the food eaten.

The present tolerances for piperonyl butoxide vary from exempt from the requirement of a tolerance (when applied to growing crops in accordance with good agricultural practice) to 8 to 20 p.p.m. for post harvest application to various vegetables, grain crops, tree nuts and uses in or on certain processed or semiprocessed food or feed products.

The effect of pesticidal synergists on bile acids and cholic acid excretion in the bile was examined by Fishbein *et al.* (1967a, 1967b, 1967c) after intravenous injection of MDP synergists into male rats. No discernible effect in bile acid or cholic acid concentration was noted after safrole injection. However, Tropital and piperonyl butoxide did alter the bile acids and cholesterol level in the bile, sometimes resulting in a concentration increase with time after synergist administration.

Another important source of MDP derivatives is sesame oil. In addition to its use in food (Budowski and Markley, 1951) it has been employed in cancer research as a lipid vehicle for other agents in carcinogenesis studies. The cocarcinogenic activitiy of sesame oil in sarcoma production was described by Morton and Mider (1939), Dickens and Weil-Malherbe (1942), and Peacock and Beck (1938). Separation of the nonsaponifiable fractions led to the isolation of sesamin, found to be cocarcinogenic by DeOme *et al.* (1949) when tested in conjunction with 3,4-benzpyrene (benzo[*a*]pyrene). Cocarcinogenic fractions were also isolated by Bischoff (1957). A weak, carcinogenic effect on subcutaneous injection into mice was also described for heated sesame oil (Steiner *et al.*, 1942) and for sesamol fed to rats (Ambrose *et al.*, 1958).

Several other MDP compounds are either carcinogenic when fed to test animals at high levels over a prolonged period of time or are capable of acting as cocarcinogens when tested in conjunction with carcinogens. Safrole is a rat hepatocarcinogen following long-term feeding at the 0.5- and 1-percent dietary levels. In both male and female rats fed safrole in a riboflavin-deficient diet, hepatic damage and nodular degeneration are produced in a much shorter time than by the deficient diet alone. The hepatic damage in males and to a lesser extent in females, under these conditions, is also manifested by marked fibrosis and massive deposits of ceroid pigment (Long *et al.*, 1963; Homburger *et al.*, 1962). Feeding of dihydrosafrole initiates cancer of the esophagus in rats (Long and Jenner, 1963). Fatty livers have been found in animals fed myristicin (Christomanos, 1927), a constituent of nutmeg, which is used as a food additive and which also possesses psychopharmacologic properties (Truitt *et al.*, 1961; Shulgin, 1966). There is no evidence for psychotropic activity with any of the commercial synergists for insecticide chemicals. The tumorigenic potential of MDP synergists is not fully clarified in the light of recent findings. The results to date with mice treated at very high dosages by subcutaneous injection or feeding for 18 months are inconclusive and further evaluation is recommended (Innes *et al.*, 1969; Falk, 1969). Several MDP compounds produced malignant tumors of the lymphatic system; these include piperonyl butoxide, sulfoxide, and *N*-propyl isome. As controls in this study, safrole and dihydrosafrole were found, as expected, to produce liver tumors, but isosafrole was not very potent in this respect. It is important to note, however, that no liver tumors were observed in rats fed piperonyl butoxide for 18 months at high dose levels (1,000, 10,000, and 20,000 p.p.m. in the diet) (Falk 1969). See Bionetics Report, J. Nat. Cancer Inst. 42: 1101–1114, 1969.

There is evidence that piperonyl butoxide and certain other MDP synergists, at high dosage, have cocarcinogenic activity and synergize the toxicity and certain pathological effects of other toxicants. The first of these reports was presented by Falk *et al.* (1965) on the cocarcinogenic potential of piperonyl butoxide and sulfoxide synergist and their interference with the detoxification and elimination of the potent environmental carcinogen, 3,4-benzpyrene. Administration of these two MDP compounds by the oral, intraperitoneal, or intravenous routes interfered with the rapid elimination of a radiolabeled sample of 3,4-benzpyrene, following intravenous injection of the latter. Detoxification of the carcinogen and biliary excretion were also decreased, as was the total recovery of the administered radioactivity. The inability to recover the administered radioactivity from treated rats as compared to untreated rats prompted the suggestions that the carcinogen was not metabolized normally and that carcinogenic activity might thus be increased. It was also established that the increased retention and activity of the carcinogen was a result of hepatic damage due to administration of 3,4-benzpyrene and the MDP synergist, and hepatic damage was correlated with alteration of a specific detoxification enzyme(s).

In a recent study by Kimbrough *et al.* (1968), the combined effect of DDT, pyrethrum, and piperonyl butoxide on pathological changes in rat liver was examined. Cytological observations established that pyrethrum produced enlargement, margination, and cytoplasmic inclusions or lipospheres in the liver cells of rats. The severity of these pathological changes was increased when piperonyl butoxide was included in with the pyrethrum. These effects were proportional to dosage and similar in character to changes caused by DDT alone. When DDT and pyrethrum were given in combination, the changes were greater than when either was given separately at the same dosage. When given at high dosages, a combination of DDT, pyrethrum and piperonyl butoxide produced characteristic liver changes just as rapidly as has been reported for DDT alone. An additive effect on liver pathology was observed when rats were fed DDT at a dietary level of only 50 p.p.m. and were kept in a room where a pyrethrum aerosol (which could contain up to 0.8 percent piperonyl butoxide) was employed at a moderate rate only once every 2 weeks, a commonly employed procedure for pest control in animal rooms. It was also concluded that variation in the extent of using synergized pyrethrum (aerosol sprays) at different times has an effect on the variation of the extent of liver cell changes observed by other investigators.

Similar studies were conducted to explore the synergistic toxicity and carcinogenicity of "Freons" and piperonyl butoxide (Epstein *et al.*, 1967b). "Freons" are fluorocarbons, with 1 to 4 carbon atoms, fluorine and sometimes chlorine, bromine or hydrogen, which are widely used as propellants with pressurized aerosols of foods or pesticides. Generally, "Freons" have low toxicity after acute or chronic inhalation exposures, with one or two exceptions. In the experiments reported, solutions were prepared in redistilled tricaprylin, containing the "Freon" (10 percent, v/v) and piperonyl butoxide (5 percent, v/v). A high mortality was consistently observed (46–55 percent) when preweaned mice (7 days old) were treated subcutaneously with the "Freon" in conjunction with piperonyl butoxide; the corresponding control mice had a lower mortality (14 percent) as did the groups receiving the test substances separately. A 15-percent mortality was observed after administration of piperonyl butoxide and 2–11 percent from "Freon" treatment. This high mortality for the combination treatment groups appears synergistic in view of the absence of such mortality attributed to piperonyl butoxide alone.

In a separate expriment to ascertain any pathological damage which might occur from administration of "Freons" and piperonyl butoxide simultaneously, weaned mice (1 month old) were treated and the liver tissue was examined cytologically at various times after treatment. All treated males displayed hepatomata 51 weeks after treatment with "Freons," although no tumors were obsreved in any of the test animals treated with piperonyl butoxide. The incidence of hepatomata was highest for combination treatment groups and particularly high for the combination involving "Freon" 112 (1,1,2,2-tetrachloro-1,2-difluoroethane) and piperonyl butoxide as compared with the solvent controls or groups receiving separate treatments of "Freons" or piperonyl butoxide. With male mice more than 40 weeks old, the incidence of hepatoma in combination treatment groups was 24 percent in contrast to an overall incidence of only 4 percent in the groups receiving individual treatments. This striking difference was shown statistically to be highly significant, and indicates that synergistic hepatocarcinogenicity results from the combination treatment. The incidence of malignant lymphomata was low and only one female developed mammary carcinoma.

The above report of synergistic toxicity and carcinogenicity between two widely used and unrelated compounds suggested the need to consider interactions with unrelated agents in the designs of toxcity and carcinogenicity tests. An extension of the findings on synergistic toxicity of piperonyl butoxide and other compounds was reported by Epstein *et al.* (1967a). Enhancement by piperonyl butoxide of the acute toxicity due to "Freon" 112 and "Freon" 113 (1,1,2-trichloro-1,2,2,trifluoroethane) with 3,4-benzpyrene and griseofulvin (an antifungal antiobotic) was examined. Mortality was generally highest in the first week of life and, in all instances, was markedly enhanced by combined treatment with piperonyl butoxide. This increased toxicity with the various synergist and drug combinations was accompanied by anomalous weight increase in surviving mice, generally being pronounced by 21 days. The authors speculated on the possibility of a toxic hazard, either synergistic or additive in nature, due to piperonyl butoxide administration in conjunction with other drugs or environmental pollutants, which should be further investigated.

Research on the ability of MDP compounds to synergize the toxicity of insecticide chemicals or drugs in mammals is sparse. The joint oral administration of piperonyl butoxide increased the toxicity of Coumaphos (0- [3-chloro-4-methyl-umbelliferone] 0,0-diethyl phosphorothioate) and its corresponding phosphate to mice fourfold to sixfold. The same increase in toxicity was also found when the synergist and toxicant were administered by different routes (Robbins *et al.*, 1959). This is the only reported case of synergism of insecticide toxicity by MDP compounds in mammals. The effects of piperonyl butoxide and sesamex on the duration of sleep induced in mice by hexobarbital were examined by Fine and Molloy (1964). They demonstrated that the synergists prolong sleep which is induced by the barbiturate. Essentially the same results were obtained when either synergist was injected simultaneously with the barbiturate. Piperonyl butoxide was also found to extend the sleeping time of another barbiturate, sodium pentobarbital (Nembutal). Anders (1968) confirmed the effect of piperonyl butoxide on hexobarbital sleeping time in rats and showed that the *in vivo* metabolism of this barbiturate is also inhibited by piperonyl butoxide, sesamex and Tropital. Zoxazolamine paralysis time is also prolonged in mice by piperonyl butoxide, although the synergist effect is less dramatic than on hexobarbital sleeping time (Fujii *et al.*, 1968).

The information available up until 1966 on the metabolism of MDP compounds was insufficient to clearly define the fate of the MDP moiety or to establish the major pathways of metabolism of MDP synergists in mammals. Mammalian metabolism of MDP compounds results in modification of the side chain. Piperonylglycine, the glycine conjugate of piperonylic acid, appears in human urine following feeding of safrole and isosafrole (Hefter, 1895); piperonylic acid is a metabolite in dog urine following administration of these two compounds. Piperonal is excreted by rabbits as the ester glucuronide of piperonylic acid: piperonylic acid is excreted by rabbits as its ester glucuronide and glycine conjugate (Williams, 1959). In rats, piperonylic acid is excreted as the free acid and its glycine conjugate, 3,4-methylenedioxycinnamic acid is excreted mostly as piperonylglycine along with some 3,4-methylenedioxycinnamoylglycine, and piperic acid is excreted as piperonylic acid, piperonylglycine, and 3,4-methylenedioxycinnamoylglycine, with no piperonylglycine (Acheson and Atkins, 1961). Ester hydrolysis occurs on oral administration of 6-chloropiperonyl chrysanthemumate (barthrin) to rats and rabbits and the liberated 6-chloropiperonyl alcohol is converted to the acid, the glycine conjugate, and the glucuronide of the acid (Ambrose, 1963; Masri *et al.*, 1964). A massive oral dose of undiluted piperonyl butoxide is excreted in the feces of dogs to the extent of 78–88 percent within 48 hours, the urine containing little more than trace amounts of piperonyl butoxide as determined by colorimetric analysis (Sarles and Vandergriff, 1952).

Fishbein and coworkers have utilized thin-layer chromatography to determine the rate of appearance and nature of the metabolites of safrole, isosafrole, dihydrosafrole, Tropital, and piperonyl butoxide in rat bile and urine following intravenous administration (Fishbein *et al.*, 1967a, 1967b, 1967c, 1968). Analysis of bile revealed the following metabolites after administration of the respective compounds: safrole-10 metabolites, five being MDP compounds (positive to chromotropic acid reagent); isosafrole-7 metabolites, two or three responding to chromotropic acid reagent; dihydrosafrole-1 chromotropic acid-positive material and two additional metabolites not containing the MDP grouping. Analysis of urine revealed a slightly different pattern as follows: safrole-5 metabolites similar to the ones detected in the bile, one responding to chromotropic acid reagent; isosafrole-3 metabolites responding positively and three negatively to chromotropic acid; dihydrosafrole-1 metabolite responding positively and four others negatively to the chromotropic acid reagent. Metabolites of safrole, isosafrole, and dihydrosafrole were not identified although oxidation of the side chain to homopiperonylic acid and piperonylic acid was postulated for safrole and isosafrole, respectively, as was cleavage of the MDP moiety to the corresponding catechols. Tropital and piperonyl butoxide were converted to unidentified materials and eliminated largely in the bile, but more slowly than the compounds previously discussed. Methylene-C^{14}-dioxyphenyl labeled Tropital yields 14 metabolites in bile, some persisting for 560 minutes, including piperonal as the major metabolite, and six other metabolites responding to chromotropic acid reagent. The urine contains piperonylic acid as a minor metabolite, in addition to one other major metabolite and one other minor metabolite, plus material remaining at the origin on chromatography. No free Tropital was excreted in the urine. The metabolites of piperonyl butoxide constitute a complex mixture involving possibly nine MDP compounds in the bile and 11 in the urine plus nine catechols or their further degradation products in the bile and 13 in the urine.

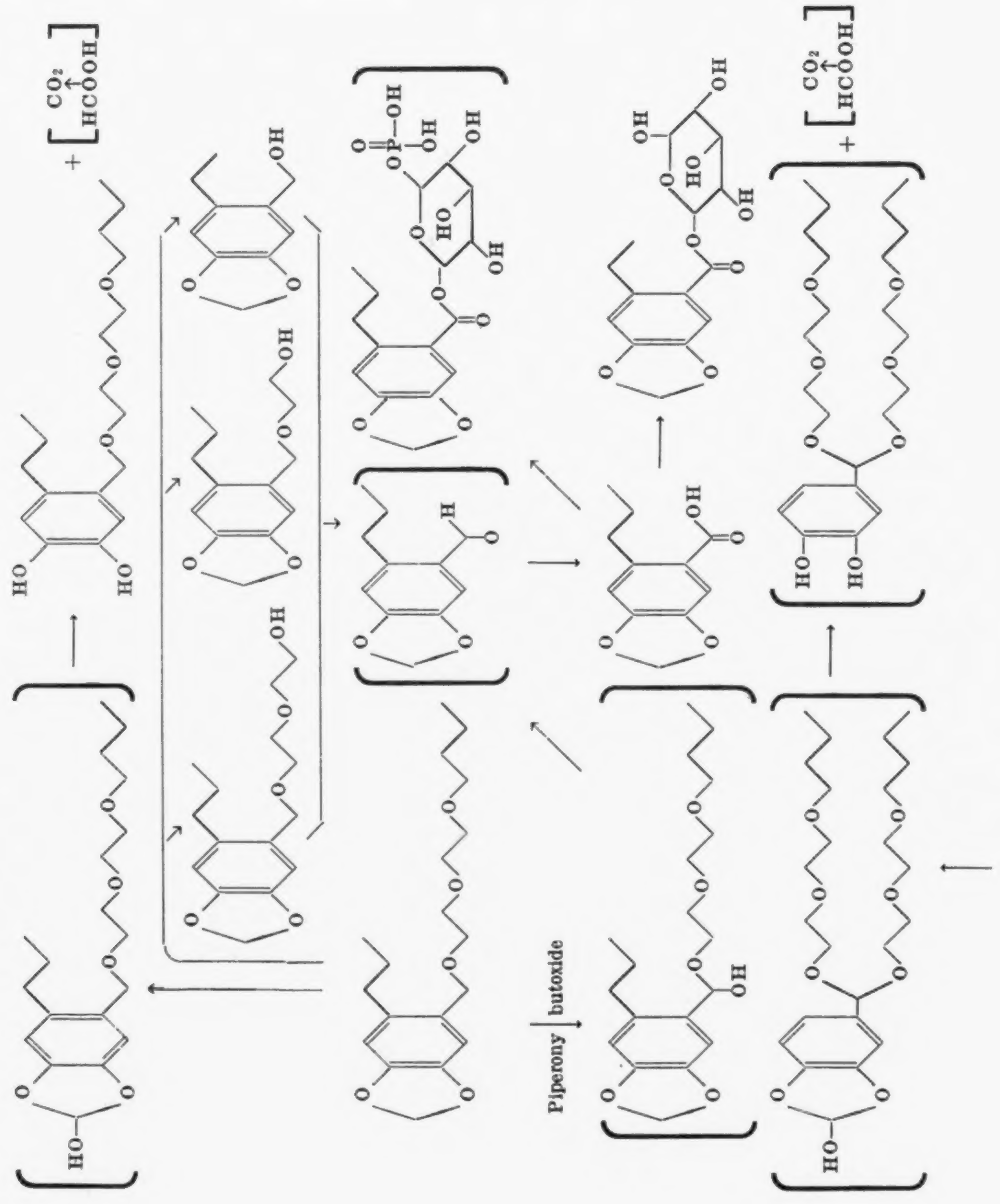
Microsomal preparations from both mammalian liver and insect tissues metabolize tetrachloromethylenedioxybenzene, in a reduced nicotinamideadenine dinucleotide phosphate (NADPH)-dependent reaction, to the corresponding tetrachlorocatechol (Wilkinson, 1967; Wilkinson and Hicks, 1969).

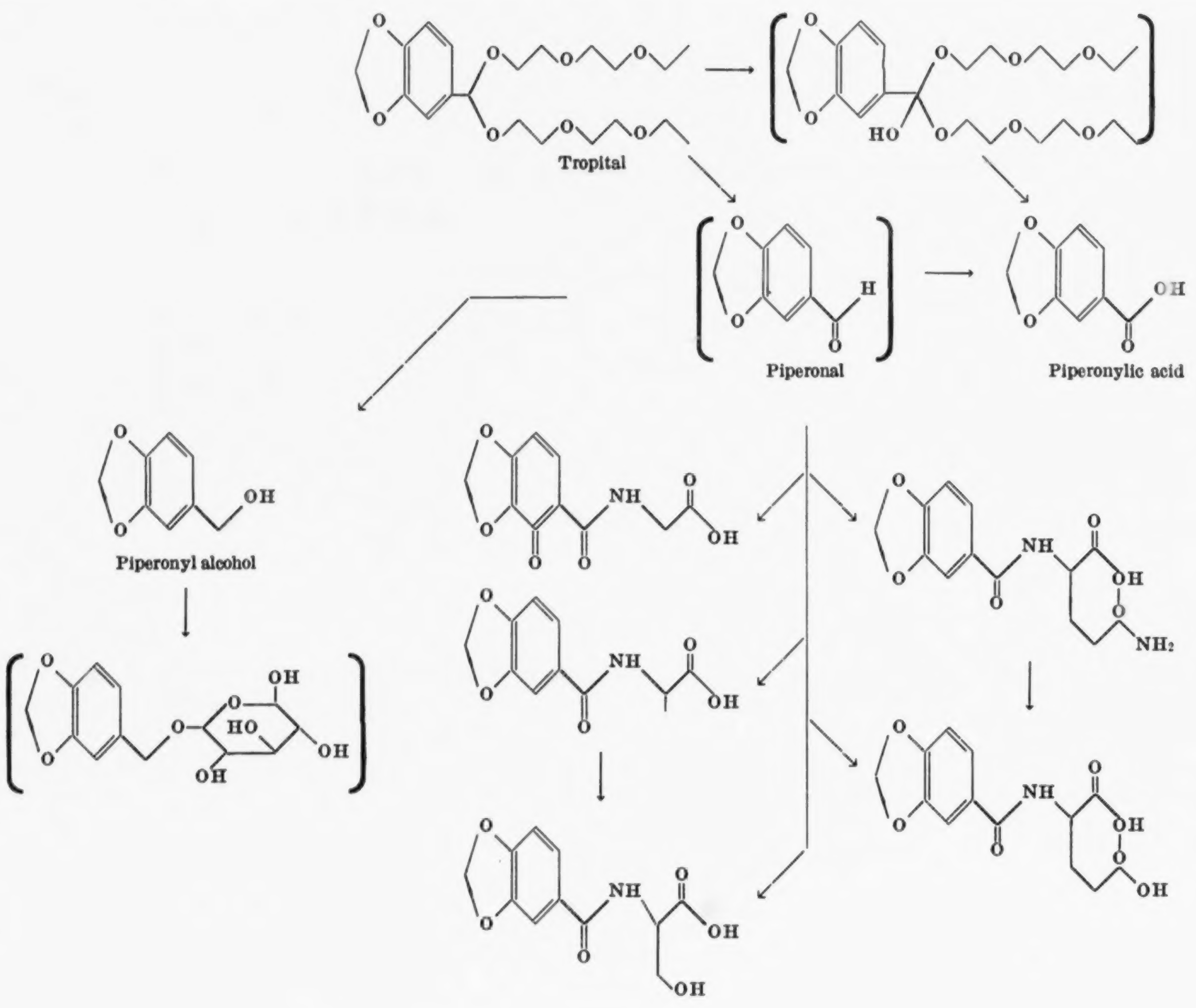
The above discussion does not consider the results of studies made at Berkeley using C^{14}-labeled MDP compounds. Prior to the synthesis of several methylene-C^{14}-dioxyphenyl (M-C^{14}-DP) compounds (Kuwatsuka and Casida, 1965), suitable analytical approaches were not available for establishing the fate, *in vivo* and *in vitro*, of the critical methylenedioxy portion of the molecule. These labeled materials were used to ascertain the fate of MDP compounds in mammals and house flies and enzyme preparations derived from these organisms.

The major metabolic pathway for piperonyl butoxide, the sulfoxide diastereoisomers, dihydrosafrole, safrole, and myristicin in mice, after oral administration, involves cleavage of the MDP moiety and expiration of the methylene carbon as carbon dioxide. In contrast, oxidation and/or conjugation of the side chain is the major metabolic pathway for Tropital, piperonal, piperonyl alcohol, and piperonylic acid. Products in the urine, following piperonyl butoxide administration, include many compounds lacking the MDP moiety along with small amounts of 6-propylpiperonylic acid and its glycine conjugate, and those from Tropital consist almost entirely of the glycine and glucuronic acid conjugates of piperonylic acid. Mixed-function oxidases of liver microsomes demethylenate several MDP compounds to yield formate and the corresponding catechol; with most MDP compounds, other products also form because of additional oxidation reactions at other functional groups (Casida *et al.*, 1966; Kamienski and Casida, 1969).

The tentative metabolic pathways found for piperonyl butoxide and Tropital in mammals are given in figures 1 and 2, respectively.

The details of the portion of the study on the metabolism of the same labeled MDP compounds in house flies has recently been published (Esaac and Casida, 1968, 1969). It was clearly shown that most of these compounds are oxidatively metabolized in living house flies by attack at the methylene carbon in the M-C^{14}-DP moiety, leading ultimately to expiration of $C^{14}O_2$. Aliphatic side chain oxidation resulted in the formation of the corresponding piperonylic acid derivatives, followed by conjugation and excretion. Following injection of piperonal, piperonyl alcohol, safrole, and Tropital, each MDP compound was oxidized to piperonylic acid which was then converted into five *N*-piperonyl amino acids, namely, the alanine, glutamine, glutamate, glycine, and serine conjugates. In addition, cleavage of the polyether side chain of piperonyl butoxide was observed, presumably mediated by hydroxylation of the methylene carbons adjacent to the ether oxygens by the microsomal-NADPH enzyme system. Summaries of the metabolism of piperonyl butoxide and Tropital in house flies are as follows: (See page 550.)


Piperonyl butoxide
HO
OH
CO₂
HCOOH
H
O
HO–P–OH


Tropital
Piperonal
Piperonylic acid
Piperonyl alcohol

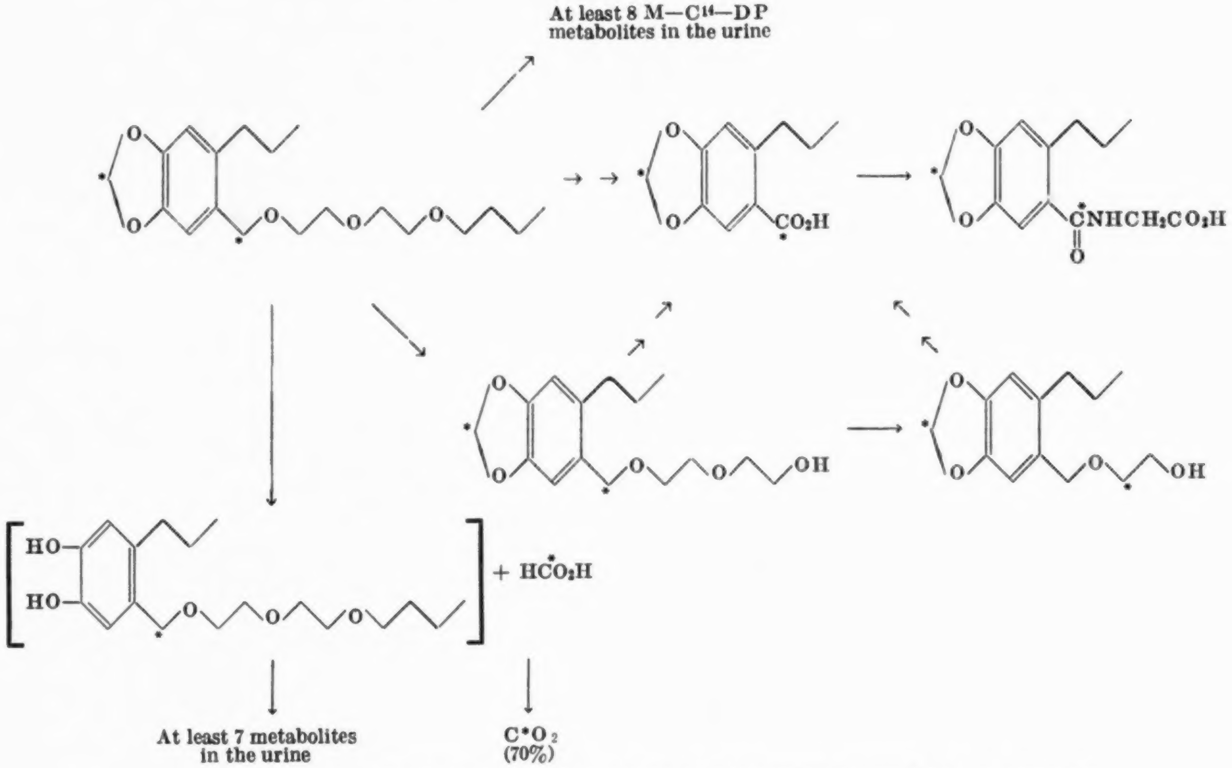

At least 8 M—C¹⁴—DP metabolites in the urine
CO₂H
CNHCH₂CO₂H
O
OH
OH
HO
HO
O
+ HC*O₂H
At least 7 metabolites in the urine
C*O₂ (70%)

FIGURE 1.—*Tentative metabolic fate of piperonyl butoxide in mammals.*

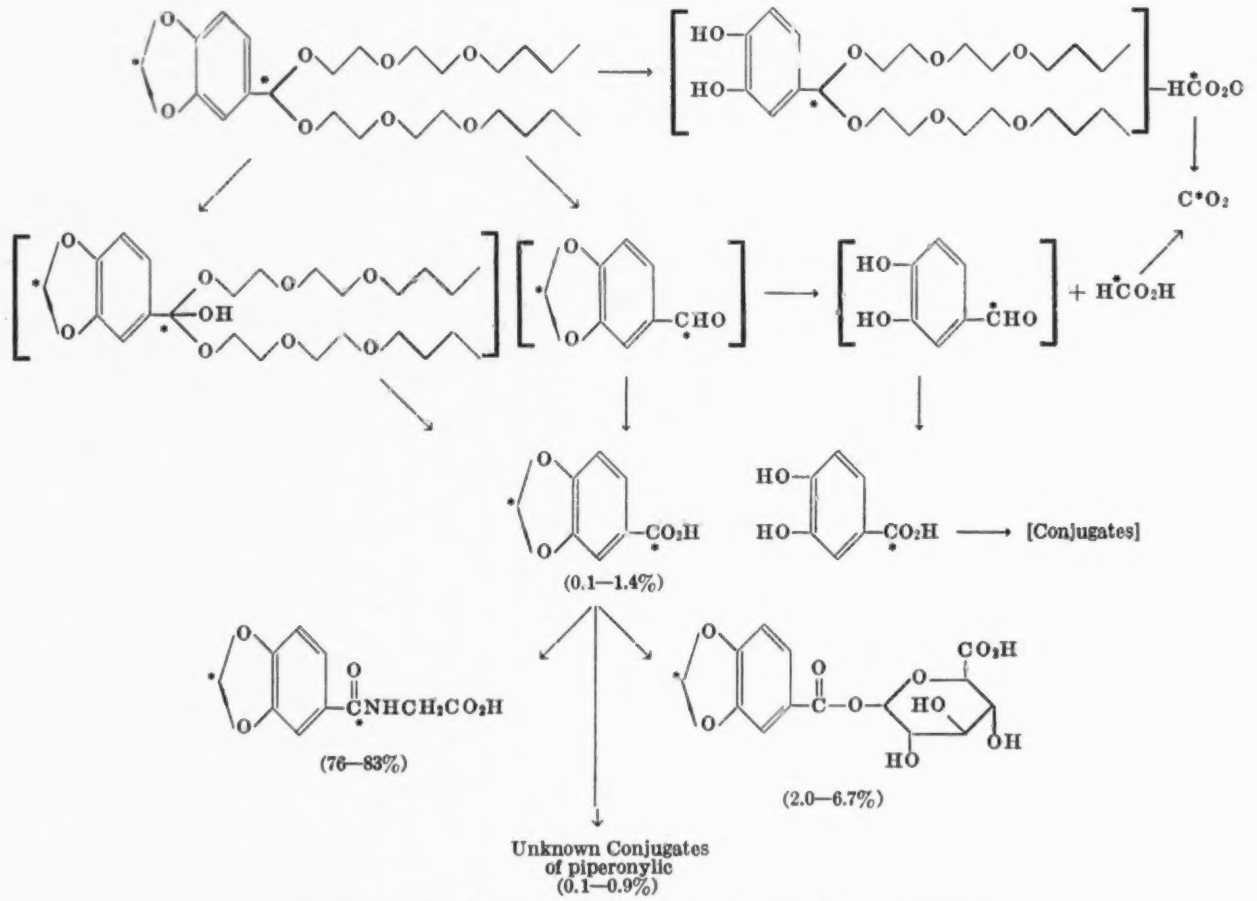

-HC*O₂O
C*O₂
OH
+ HC*O₂H
CHO
CO₂H
[Conjugates]
(0.1—1.4%)
CNHCH₂CO₂H
(76—83%)
CO₂H
HO
OH
(2.0—6.7%)
Unknown Conjugates
of piperonylic
(0.1—0.9%)

FIGURE 2.—*Tentative metabolic fate of tropital in mammals.*

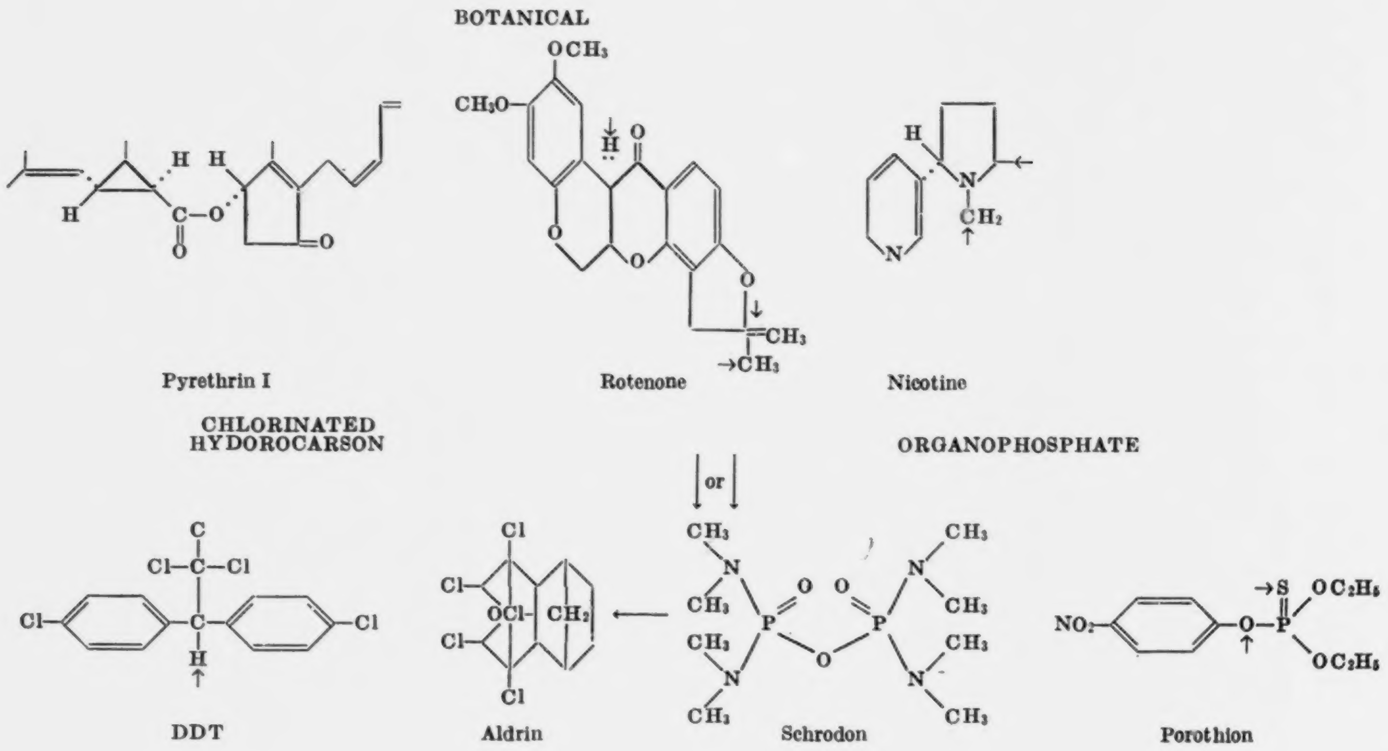
In both insects and mammals, sulfoxide synergist undergoes demethylenation and oxidation at the sulfur (Esaac and Casida, 1969; Kamienski and Casida, 1969). The comparison of synergist metabolism with the fate of insecticide chemicals was recently reviewed (Lykken and Casida, 1969).

MODE OF ACTION OF METHYLENEDIOXYPHENYL COMPOUNDS

Studies with both insects and mammals have aided in formulating hypotheses regarding the mode of action of MDP compounds. In earlier proposals, synergistic action was supposedly due to stabilization of the toxicant or formation of molecular complexes between the synergist and the insecticide (Metcalf, 1955), but these proposals have been discarded for lack of experimental evidence. Presently, the most widely accepted hypothesis to explain the mode of action of MDP synergists is that they act by inhibiting the detoxification of the insecticide chemical in insects (Casida, 1963; Metcalf, 1967) or by reducing the rate of drug detoxification in mammals (Fine and Molloy, 1964). These drugs or insecticides are metabolized almost exclusively by the NADPH-dependent mixed-function oxidase system of microsomes. The oxidative reactions mediated by liver and insect microsomes have been extensively reviewed by Gillette (1963) and Casida (1969).

In a more specific manner, synergists are proposed to exert their effect by blocking oxidative detoxification reactions, presumably by serving as alternative substrates (Casida *et al.*, 1966; Wilkinson and Hicks, 1969) or competitive inhibitors (Philleo *et al.*, 1965) for the microsome-NADPH enzyme system, so that a lower initial dose of the toxicant is effective. The inhibition of rat liver microsomal mixed-function oxidases by piperonyl butoxide was competitive with two substrates, but proved to be variable with other MDP compound-substrate combinations and so generalization is not possible (Anders, 1968); difficulties in obtaining clear-cut interpretations from kinetic studies of this type have also been encountered by many other workers. Jaffe *et al.* (1968) assayed the important MDP synergists and many related compounds for *in vivo* inhibition of two hydroxylating systems of mouse liver microsomes, using dimethylaminopyrine and hexobarbital as substrates. The structure-activity correlations were similar to those for insecticidal synergism, indicating that synergists and related compounds are probably of comparable toxicological significance to mammals. The inhibition appeared, in general, to be relatively nonspecific in character. These findings, combined with those of Kamienski and Casida (1969) discussed above, suggest a possible correlation, at any given time after administration, between the extent of demethylenation (or perhaps, as well, of other microsomal hydroxylation reactions acting on the MDP compound) and the *in vivo* inhibition of microsomal hydroxylations of other substrates. In utilizing this hypothesis, consideration must be given to the relative biological stability of the MDP compound and the alternative substrate, and to the inhibitor and substrate specificities of the liver mixed-function oxidases system(s) (Kamienski and Casida, 1969). The effectiveness of piperonyl butoxide as an *in vivo* and *in vitro* inhibitor of drug metabolism in rats is markedly diminished by chronic treatment with either phenobarbital or 3-methylcholanthrene, possibly because these agents induce rapid metabolism of piperonyl butoxide to products having minimal inhibitory capacity (Anders, 1968). Piperonyl butoxide serves as an inducer of biphenyl metabolism, by both *o*- and *p*-hydroxylation (Jaffe *et al.*, 1969). In a separate and distinct phenomenon from the induction, piperonyl butoxide stimulates *o*-hydroxylation and inhibits *p*-hydroxylation of biphenyl by liver microsomes of treated mice, a bimodal effect occurring shortly after treatment which may represent an isozymic transformation shifting the equilibrium between the two microsomal enzyme activities (Jaffe *et al.*, 1969b); this bimodal effect also occurrs *in vitro* (Jaffe *et al.*, 1969a).

MDP compounds have been shown to inhibit or retard, among others, the following oxidative reactions in both insects and mammals necessary for detoxification or inactivation of the chemical in question: oxidation of the *trans*-methyl group of the isobutenyl moiety of pyrethrin I and synthetic chrysanthemumates to the corresponding carboxylic acid analogs in living housefles and isolated enzyme systems (Yamamoto and Casida, 1966; Yamamoto *et al.*, 1969); *in vitro* hydroxylation of *N*-methyl groups of *N*-methy land *N,N*-dimethylcarbamates by both insect preparations (Tsukamoto and Casida, 1967a, 1967b) and rat liver enzymes (Hodgson and Casida, 1960, 1961; Leeling and Casida, 1966); *in vivo* *N*-demethylation of the *N,N*-dimethylamide group of Bidrin insecticide in houseflies (Menzer and Casida, 1965); *O*-depropylation of Baygon in living houseflies and isolated abdomen enzymes (Tsukamoto and Casida, 1967a, 1967b; Shrivastava *et al.*, 1969)ffi hydroxylation of aromatic hydrocarbons such as naphthalene by house fly microsomal enzymes (Philleo *et al.*, 1965) or the aromatic nucleus of *l*-naphthyl *N*-methylcarbamate by rat liver microsome-NADPH enzymes (Leeling and Casida, 1966); oxidation of the methyl group of toluene and *p*-nitrotoluene by housefly microsomes to the corresponding benzoic acids (Chakraborty and Smith, 1967); the NADPH-dependent conversion of phosphorothionates to the corresponding phosphate analog by American cockroach


BOTANICAL
OCH₃
CH₃O
H
O
H
H
H
H
O
C
O
O
O
O
O
CH₃
CH₃
N
N
CH₂
Pyrethrin I
Rotenone
Nicotine
CHLORINATED HYDOROCARSON
ORGANOPHOSPHATE
or
C
Cl—C—Cl
Cl
Cl
C
H
Cl
Cl
Cl
OCl
CH₂
Cl
Cl
CH₃
N
CH₃
CH₃
N
CH₃
P
O
O
P
O
CH₃
N
CH₃
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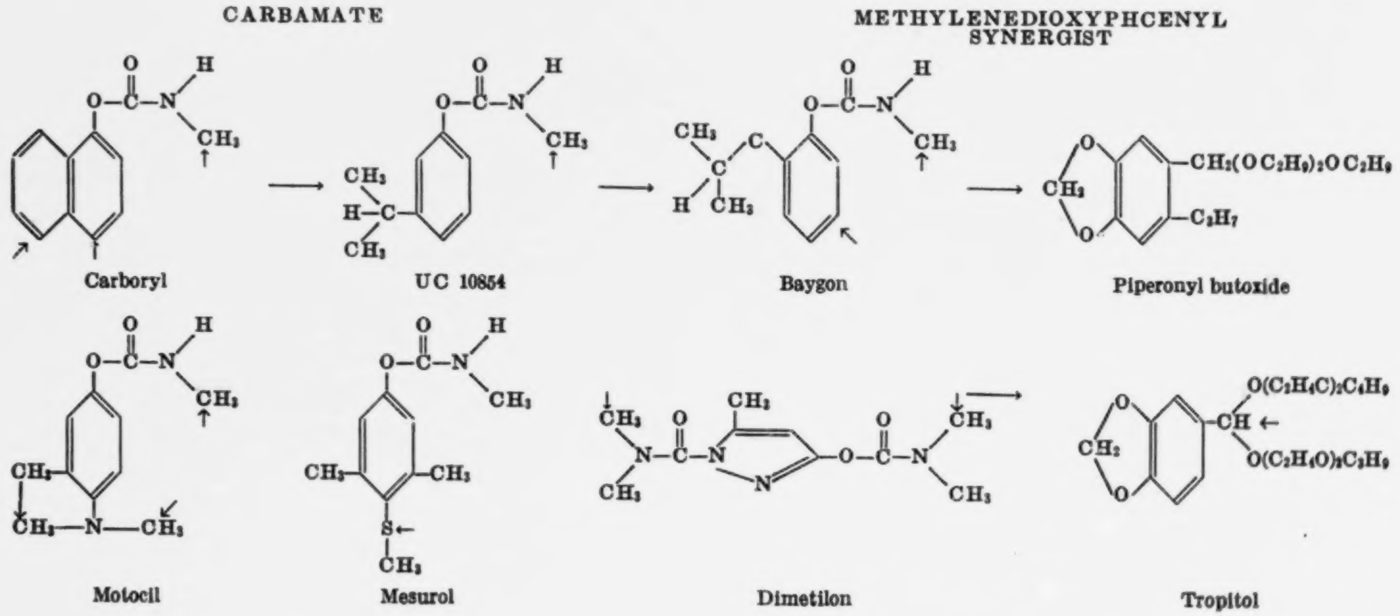
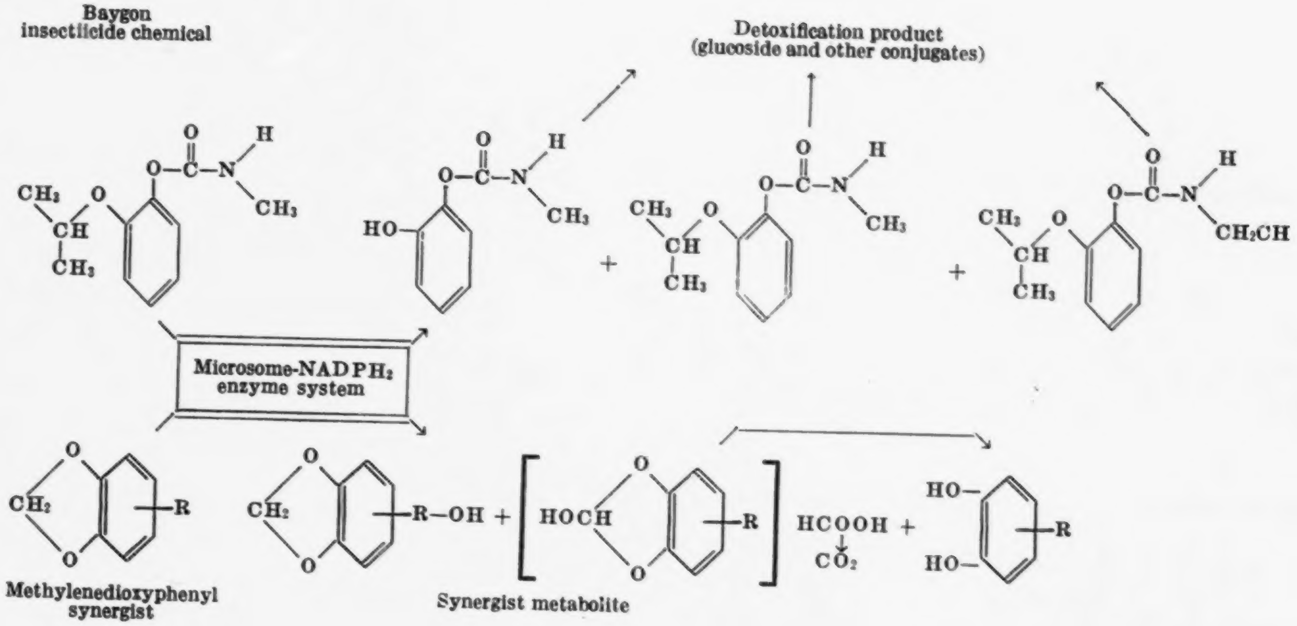
DDT
Aldrin
Schrodon
Porothion


CARBAMATE
METHYLENEDIOXYPHCENYL SYNERGIST
Carboryl
UC 10854
Baygon
Piperonyl butoxide
Motocil
Mesurol
Dimetilon
Tropitol

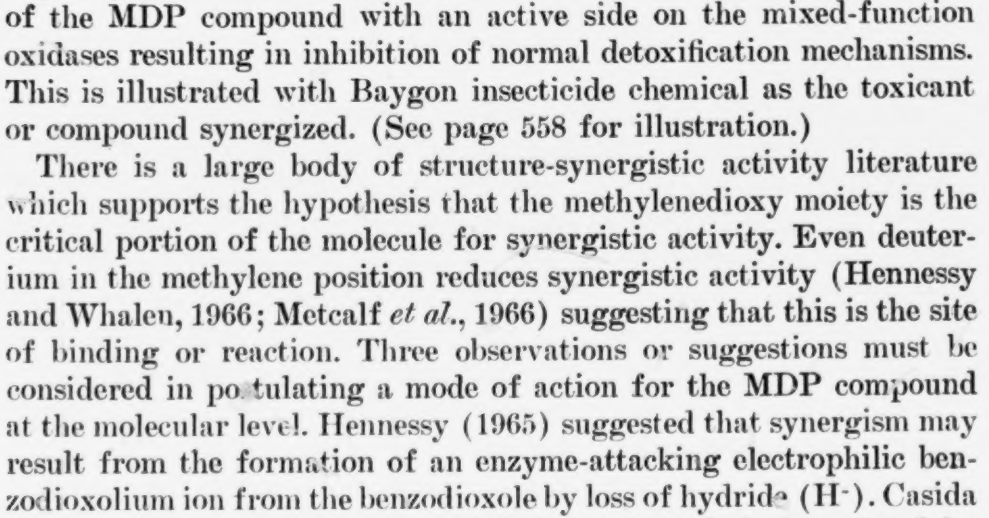
547


Baygon
insecticide chemical
Detoxification product
(glucoside and other conjugates)
Microsome-NADPH₂
enzyme system
Methylenedioxyphenyl
synergist
Synergist metabolite
HCOOH
CO₂

fat body microsomes (Nakatsugawa and Dahm, 1965) or by rat liver microsomes(Dahm *et al.*, 1962); microsomal epoxidation of cyclodiene insecticides such as the conversion of aldrin to dieldrin (Lewis *et al.*, 1967; Nakatsugawa *et al.*, 1965); and the dehydrochlorination of DDT to DDE by houseflies (Perry and Hoskins, 1950, 1951). The list of reactions, which continues to grow, is partially illustrated on pages 556 and 557. (Casida, 1969.)

The action of MDP synergists and related compounds in increasing drug and insecticide potency possibly is the result of combination of the MDP compound with an active side on the mixed-function oxidases resulting in inhibition of normal detoxification mechanisms. This is illustrated with Baygon insecticide chemical as the toxicant or compound synergized. (See page 558 for illustration.)

There is a large body of structure-synergistic activity literature which supports the hypothesis that the methylenedioxy moiety is the critical portion of the molecule for synergistic activity. Even deuterium in the methylene position reduces synergistic activity (Hennessy and Whalen, 1966; Metcalf *et al.*, 1966) suggesting that this is the site of binding or reaction. Three observations or suggestions must be considered in postulating a mode of action for the MDP compound at the molecular level. Hennessy (1965) suggested that synergism may result from the formation of an enzyme-attacking electrophilic benzodioxolium ion from the benzodioxole by loss of hydride (H^-). Casida *et al.* (1966) showed that the methylene group is hydroxylated by the mixed-function oxidase system to give a hydroxymethylenedioxphenyl intermediate; in this manner, the synergist is an alternative substrate for the enzyme. Hansch (1968) and Cloney and Scherr (1968) present physico-chemical correlations which suggest a free radical enzymatic reaction to form a MDP$^{\bullet}$ free radical and/or a reactive benzodioxolium cation. These considerations lead to two pathways or mechanisms (Kamienski and Casida, 1969; Hennessy, 1969):



Pathway A leads to direct formation of the benzodioxolium ion or accommodates the free radical mechanism in enzymatic formation of the hydroxymethylenedioxyphenyl compound, which is the pseudobase of the benzodiheterolium ion. The benzodioxolium ion as an aromatic system might form a π-bonded complex with iron or copper Also, it might acylate the enzyme, subsequently hydrolyzing to formate and the catechol. Pathway B utilizes the MDP free radical as the species reacting with the enzyme, thus:

A. $\mathrm{Fe^{++} + MDP^{+} \rightleftharpoons [FeMDP]^{+++}}$

B. $\mathrm{Fe^{+++} + MDP\cdot \rightleftharpoons [FeMDP]^{+++}}$

Kuwatsuka (1969) interprets studies of the different spectra of the P–450 hemoprotein and of the kinetics of inhibition with different substrates to indicate that the enzyme probably has different binding sites for different substrates and the enzyme and inhibitor interact in some other relationship than that of enzyme and substrate, i.e., allosteric effects are involved.

Each of these hypotheses requires that the synergist binds tenaciously or alters the enzyme, and that the synergist is metabolized more slowly than the insecticide. Insect microsomes are frequently more sensitive than mammalian microsomes to MDP compounds (Lewis *et al.*, 1967) and metabolize the synergist more slowly (Esaac and Casida, 1969). The mechanism of reaction of the enzyme and synergist studied with mammalian liver and house fly microsomes, for the most part, may be found to differ between insects and mammals. Further studies are needed, with MDP and other synergistic compounds, to determine the mechanism involved in the higher sensitivity of insect then mammalian mixed-function oxidases to inhibition.

LEVELS OF EXPOSURE TO MDP COMPOUNDS

MDP compounds are natural dietary constituents and potential contaminants from the use of insecticide synergists. Certain of the natural MDP compounds as well as the MDP synergists act in mice at high dosages to give transient inhibition of liver microsomal mixed-function oxidases (Csillag *et al.*, 1969). The human dietary level of these materials, individually or collectively, is either unknown or is not in the readily available literature. However, data on the residues of synergists which are in use or are proposed for use are part of the petitions for tolerances or exemptions submitted to the USDA. There is little if any precise data on human intake from domestic use of piperonyl butoxide-containing formulations. The chemistry of piperonyl butoxide degradation on exposure to ambient environmental conditions is poorly understood at present, although there is evidence that the MDP synergists are photochemically unstable to sunlight. (Casida, 1969.)

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CHAPTER 7

Mutagenicity of Pesticides

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MUTAGENICITY OF PESTICIDES

SUMMARY AND CONCLUSIONS

Among the plethora of new chemicals in our increasingly complex environment, a number are already known to be mutagenic, *ie.*, capable of producing genetic damage. When genetic damage occurs, the burden of hereditary defects in future generations is increased.

One potential genetic hazard comes from pesticides. Although we can point to no pesticide now in wide use that has been demonstrated to be mutagenic, the overwhelming majority have, however, not been adequately tested, although appropriate methodologies are now available.

We define mutations as any inherited alteration in the genetic material. Such alterations in exposed individuals may lead to cancer and to teratological effects. Our main concern, however, is for their descendants; for such changes lead to a wide range of abnormalities, mental retardation, physical and mental disease, and all the other inherited weaknesses and debilities to which man is susceptible. Since these effects will occur in future generations and may be apparent only many generations removed, by the time the effect is noticed, the damage is already irreversible. It is therefore urgent that any mutagenic chemicals to which the population is exposed be promptly identified.

There are now about 400 substances that, in various forms and combinations, are currently used as pesticides. It is feasible to test all of these in the near future for mutagenicity in systems that are simple and precise and yet relevant to man.

For these and other reasons detailed in the report, we recommend that:

a. All currently used pesticides be tested in the near future in four systems (as indicated on p. 602). Pesticides should be tested at concentrations substantially higher than those to which the human population is likely to be exposed. Test procedures should reflect routes of human exposure. Apart from the obvious route of ingestion, particular and critical attention should be directed to the inadequately appreciated route of inhalation, especially for pesticide aerosols and for vaporizing pesticide strips which are widely used domestically.

b. Pesticides found to be inactive in all these tests may be regarded as safe, unless other evidence of mutagenicity appears. Use of mutagenic pesticides must be rigorously restricted or banned unless thorough and impartial study demonstrates convincingly that the benefit outweighs the risk.

c. No new pesticides should be registered until tested for mutagenicity.

d. Some disinterested scientific group or commission should be charged with responsibility for continued surveillance of the whole problem of pesticide mutagenesis.

IMPORTANCE OF MUTAGENICITY AS A PUBLIC HEALTH HAZARD

A particular subtle danger from wide scale use of pesticides lies in the possibility that some of them may be damaging to the hereditary material. If this is so, we may be unwittingly harming our descendants. Whether this is happening, and if so, what is the magnitude of the effect, is regrettably unknown. Surely one of the greatest responsibilities of our generation is our temporary custody of the genetic heritage received from our ancestors. We must make every reasonable effort to insure that this heritage is passed on to future generations undamaged. To do less, we believe, is grossly irresponsible.

The first evidence that environmental agents under human control might have some influence on the genetic constitution of future populations followed the discovery that high energy radiation causes mutations. The first convincing evidence of this came with the publication in 1927 of M. J. Muller's classical paper "Artificial Transmutation of the Gene." Muller was quick to point out the potential health hazard associated with indiscriminate use of radiation.

The discovery of nuclear energy brought a whole new dimension to the problem and a greatly increased public awareness of genetic hazards. Out of this concern, originally confined largely to geneticists, radiologists, and radiation biologists, but later including persons of a great diversity of special interests, have come rigorous safeguards to insure that radiation exposure is kept to the lowest practicable minimum.

As soon as radiation-induced mutagenesis was discovered, there were strong reasons to suspect that many chemicals would have the same effect, but proof of this did not come until World War II when mustard gas was shown to induce mutations in fruit flies. Since that time, very efficient test systems have been developed and a large number of chemicals of a great diversity of structure and activity have been shown to be mutagenic. The likelihood that some highly mutagenic chemicals may come into wide use, or indeed may already be in wide use, is great enough to be a cause for real concern.

Pesticides are only a part of the plethora of new chemical compounds that have become a part of our environment, but they are of particular concern because they are used so widely and in such enormous amounts. Furthermore, they are very potent biologically; if they weren't they would not be effective pesticides. Although the mechanisms by which most pesticides kill, or inhibit growth, or sterilize the various animal and plant pests for which they are designed is thought to be unrelated to genetic mechanisms, our ignorance of chemical mutagenesis will not allow the assumption of safety without specific mutagenic tests.

What are mutations and what effects will they have on the human population? In its broadest usage, the word mutation is used to designate any inherited change in the genetic material. This may be a chemical transformation of an individual gene that causes it to have an altered function. Or the change may involve a rearrangement, or a gain or loss, of parts of a chromosome. This kind of change is often visible by ordinary microscopy. We shall use the word gene or point mutation to designate changes of the individual gene and speak of those changes which involve the larger chromosomal units as chromosome aberrations. In many experimental systems, these are easily distinguished, but in human studies, classification of an individual defect, as to whether it is due to a point mutation or a chromosome aberration, is not always possible.

Mutations may occur anywhere in the body. Frequently, the result is the death of the particular cell in which the change occurs. Most of the time this causes only local and transient damage, for most individual cells are quite dispensable. But if the change is of such a nature as to change the genetic functioning of the cell while still permitting it to divide, this change may be transmitted to descendant cells and the damage is then less localized. The effect may be cancer or it may be teratogenic; particularly if the change takes place during embryonic development. We are especially interested, of course, in those changes that occur in the germ cells—cells that are the progenitors of future generations. A mutation or chromosome change that is transmitted *via* the sperm or egg to the next generation can effect every cell in the body of the descendant individual, with consequences that may be disastrous.

What kinds of effects on the human being do mutations produce? Perhaps the most important fact to emphasize is that there is no single effect. Since every part of the body and every metabolic process is influenced by genes to a greater or lesser extent, it comes as no surprise that the range of effects produced by gene alterations includes every kind of structure and process.

At one extreme are consequences so severe that the individual cannot survive, so-called lethal effects. If the death occurs very early in embryonic development it may never be detected. If the death is at a later stage, it may lead to a miscarriage. An appreciable fraction, very roughly one-fourth, of spontaneous abortions, shows a detectable chromosome aberration, and there is no way at present to know how many of the remainder are caused by gene mutations or by chromosome aberrations too small to detect by the microscope. If the embryo survives until birth there may be physical abnormalities. There are hundreds of known inherited diseases and probably many more that are unknown, all of which owe their ultimate cause to mutations. These are individually rare, but collectively account for a substantial fraction of human misery. And, perhaps most tragic of all, genetic factors play a role in the causation of mental deficiency and disease.

At the other extreme are genes with mild effects. Those with still smaller effects finally become imperceptible. In between these extremes are the whole gamut of minor to severe genetic defects. So, it is evident that the effect of an increased mutation and chromosome aberration rate is not something new, but rather an increased frequency of diseases, abnormalities, weaknesses, and assorted human frailties that are already occurring.

Many mutations produce effects that are similar to those produced by other, nongenetic causes. And, we must remember that spontaneous mutations are happening all the time. For all these reasons, the impact of environmental mutagens is statistical rather than unique. This problem is further complicated by the time-distribution of mutational effects. Some mutant genes are dominant, in which case, the abnormality or disease will appear in the very next generation after the mutation occurs. On the other hand, the gene may be recessive, that is to say it may require the abnormal genes in both homologous chromosomes (one derived from the male and the other from the female parent) to produce the effect. In this case, the disease or abnormality may be delayed for many generations until some unlucky child inherits a mutant gene simultaneously from each of his parents. The net effect of all this is that, although the first generation probably will manifest a larger effect than will any particular subsequent generation, the overall effect is spread over many generations. What happens in the first generation is only a fraction of the total impact of the mutation process.

That the great majority of mutations should be harmful to a greater or lesser extent (or at best, neutral), is both a deduction from the principle of natural selection and an empirical fact well established in experimental systems. In the human past, natural selection has ruthlessly eliminated those individuals whose mutant genes caused them to be abnormal, diseased, or even only slightly weakened. As a result, there has been an approximate equilibrium between the introduction of new mutant genes into the population by mutation and the elimination of old genes by natural selection. But with our present high standards of living and health care, many mutants that in the past would have caused death or reduced fertility now persist. So the equilibrium is out of balance and new mutants are being added to the population faster than they are being eliminated. This, coupled with the near eradication of many infectious diseases, means that now and in the future our medical problems will be increasingly of genetic origin.

A mutation, once it has occurred, is transmitted from parents to succeeding generations. If the gene causes a lethal or sterilizing effect, it will persist for only one generation and affect only one person. On the other hand, if it causes only a slight impairment it may be transmitted on from generation to generation and thereby affect many people. There is, therefore, generally an inverse relation between the severity of the gene effect and the number of persons that will be exposed to this effect. If it were not for this, we could dismiss as relatively unimportant the effects of mild mutants. But in any overall consideration, we must consider many persons mildly affected as being of comparable importance to one individual severely affected. Experiments on fruit flies show that mildly deleterious mutations occur with much greater frequency than do more severe mutants—at least 10 times as frequently. All this makes it likely that, although an increased mutation rate would cause a corresponding increase in severe abnormalities and genetic diseases, the major statistical impact of a mutation increase on the human population would be to add to the burden of mild mutational effects. This would make the population weaker, more prone to disease, and more likely to succumb to an effect that otherwise would be resisted.

All these implications mean that it is not possible to predict in detail the kinds of effects that would occur following an increased mutation rate, nor their distribution in time. Nor can we be at all accurate in any quantitative assessment of the total harmful impact of mutation on the population in comparison with other hazards. So, in weighing benefits against risks of possibly mutagenic pesticides, we have only a vague idea of the nature and magnitude of the risk. We must remember, however, that genetic damage is irreversible by any process that we know of now. The risk to future generations, though difficult to assess in precise terms, is nevertheless very real. The prevention of any unnecessary mutational damage is one of our most important and immediate responsibilities.

Despite the extensive use of pesticides, our information on their possible mutagenicity is grossly inadequate. Several have been tested in various test systems, but we believe that none has had the kind of systematic testing that would be regarded as adequate. Such testing, we believe, is entirely practical and feasible. There are numerous widely used test systems that are precise, efficient and relatively inexpensive. However, these mainly depend on microbial, insect, or plant systems and there is a question as to their relevance to man. For definitive testing, it is necessary to use systems that have a high degree of presumptive human relevance. We believe that such satisfactory systems now exist, that are practical, sensitive and relevant. In this report, we recommend that a combination of these be routinely applied to all pesticides.

METHODOLOGIES FOR MUTAGENICITY TESTING

A variety of methodologies are now available for mutagenicity testing. From the criterion of presumptive human relevance, they have been categorised as ancillary systems and as mammalian systems. The human relevance of data obtained from ancillary test systems is uncertain, in view of factors such as cell uptake, metabolism, detoxification, dosage, and method of administration. The mammalian systems embody fewer of these drawbacks.

Since no single method can detect all possible types of mutations, a combination of methods must be used. A positive result in *any* of the mammalian systems represents evidence of a potential mutagenic hazard. The danger inherent in the use of restricted and inappropriate test systems is apparent from recent contract-supported studies in which mutagenic activity of pesticides was tested in microbial systems. In these studies, the microbial systems could have detected only point mutations, whereas structural considerations indicated that the pesticides tested could only induce inactivating DNA alterations resulting in chromosome breaks and aberrations. Additionally, some of the pesticides required microsomal enzymatic activation which could only occur in *in vivo* mammalian test systems.

In addition to these test procedures, human population monitoring may reveal mutagenic effects of pesticides or any other environmental agents that have escaped detection.

Ancillary methods

Bacterial.—A variety of relatively simple and inexpensive tests are available for demonstrating point mutations. (*1*) However, these systems are generally insensitive to chemicals inducing chromosome breakage in higher cell forms. Reverse and forward mutations are generally tested using biochemical markers; additionally, drug resistance is used as a marker for forward mutations. These methods include:

a. Reverse mutation system in *Salmonella*. Histidine-requiring mutants exist which revert by single base pair changes, i.e., transitions or base pair insertions or deletions. By selection of the proper strains, most possible point mutation mechanisms can be detected.

b. Forward mutation systems based on resistance to streptomycin or other antibiotics, can be used. It is, however, uncertain how many places in the gene can mutate to give resistance mutants, and therefore, it is a question whether all types of base pairs changes can be detected.

c. Differential staining techniques (Eosin-Methylene blue), exist in which lactose nonfermenting mutations can be detected and quantitated.

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Neurospora.—*Neurospora crassa* is a haploid organism with seven chromosomes and a normal meiotic cycle. However, by using a balanced heterokaryon between biochemically marked strains, the diploid phase of higher organisms can be mimicked. Chromosome deletions as well as point mutations can thus be detected. Forward mutations can be recovered in the ad-3 region of chromosome 1 (*1*), without applying selective techniques. Either growing cultures or spores (*conidia*) can be exposed to chemicals under test. After the treatment, *conidia* are inoculated into 10 litre Florentine flasks and incubated for 7 days. Each flask can contain 10^6 colonies which are screened for presence of purple mutants. The frequency of the different fractions of the *conidia* population from the heterokaryon can be determined by plating on different substrates.

Very refined genetic analysis can be carried out on the mutants. The frequency and the size of the chromosome deletion can be determined (*5*), and the genetic alterations of the point-mutations can be identified at the molecular level (*3*, *4*). From the plate counts, it is possible to distinguish between nuclear and cytoplasmic inactivation.

Mutations frequencies induced by 500 r can be easily and practically detected. *Neurospora* is obviously metabolically different from mammals; therefore, tests for mutagenicity should include mammalian metabolites of the pesticide and the use of *Neurospora* in the host mediated assay system (*2*).

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Phage and transformation (*1*) : *Phages.*—Bacteriophage T_4 is probably the best available system. Forward mutations to "r" phenotype are of low sensitivity; reverse mutations of "rII"—type mutants are of high sensitivity. Chemicals inducing point mutations, which alter DNA either chemically (treatment of free phages) or during its duplication inside the bacteria can be detected. The sensitive assay of reverse mutations induction responds only to those agents which induce the required specific base pair change, e.g., $\substack{CA \\ \to TG}$. In order to detect all types of base pair changes, a set of about 6 rII mutant strains having the required base pair changes should be tested. Agents which induce only inactivating DNA alterations rarely induce point mutations. They do, however, inactivate phage, but only more detailed genetic tests can verify that the inactivation is not caused by an alteration of phage protein.

Transformation.—The ideal system is that of linked mutation induction, which at present, is limited to the induction of fluorescent mutants in the tryptophan operon. Forward mutations to fluorescence are of medium sensitivity. Reverse mutations to indole independence are of high sensitivity. In these systems, inactivating DNA alterations can be measured and quantitatively compared to mutagenic DNA alterations. It has been shown that radical producing agents, known to induce both chromosome breaks and large chromosome mutations, inactivate transforming DNA but do not induce point mutations. Thus, in most bacterial or phage systems, these agents would not induce mutations and might be erroneously labeled nonmutagenic. Only agents which directly act on resting DNA can be easily assayed. For agents, like base analogs, which induce mutations in duplicating DNA, such measurements are difficult.

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Plants.—An extensive body of literature exists on the response of higher plants to chemical mutagens and many of the techniques laboriously worked out for experiments with physical mutagens should be equally applicable to experiments with known or suspected mutagens (*30, 46, 61, 105*).

Since the literature indicates that many of the well known mutagenic and/or chromosome breaking chemicals are as effective in plant material as in animal test systems and since several of the plant systems can detect effects of such substances applied in the gaseous state (*95*), and since some, if not all, plant species are highly susceptible to chemical mutagens, appropriate plant material should be included in the battery of tests to be performed in screening or testing for mutagenicity of various chemical compounds. Further, since plant chromosomes are structurally more akin to mammalian chromosomes than are those of viruses, bacteria and other prokaryotic organisms, responses of plant chromosomes to chemical mutagens should provide valuable information with respect to their possible mutagenicity in mammals. Also, the factors determining the inherent radiosensitivity of plant cells are now fairly well understood (*102*) and this knowledge may offer valuable guidance for work with chemical mutagens.

Plant test systems include many species and a considerable variety of possible procedures at various stages of development (table 1). It is not feasible to select a specific test as the best in all possible cases. Circumstances and objectives of the experiment would determine which test and which species should be recommended.

The efficiency of the various plant test systems varies widely. However, some of the most efficient ones compete favorably with other nonplant tests and some e.g., *Tradescantia*, have the advantage that they can be used as monitors over long exposure periods. Inspection of the flowers for somatic mutations once or twice a week should reveal quickly whether or not a level of mutagen exposure has occurred. Of course, the lower the level or the shorter the exposure, the more effort is required to show a significant increase above the normal background rate. *Tradescantia* is especially sensitive to both ionizing radiation (*66*, *97*) and chemical mutagens (*95*), with the effects of a few rads being readily detectable and saturation of the somatic mutation rate occurring around 200 R of gamma rays (*72*). Some other plant systems such as somatic mutation in *Nicotiana* are also very sensitive (*86*); those in which mutations, chromosome aberrations or lethal effects are readily detectable in microspores or pollen tend to be highly efficient (table 1).

A partial list of chemical mutagens and/or pesticides known to be effective in higher plants is given in tables 2 and 3. Several other pesticides are also known to be mutagenic in plants, e.g., cytrol (*111*), hyvar (*111*), lindane (*90*), and vapona (*90*). An indication of relative mutation rates produced by gamma rays and various chemical mutagens in several test systems is given in table 4.

A brief outline of the procedures considered to be most promising for chemical mutagen studies follows. It includes methods for analyzing various types of chromosomal aberrations, mutations and lethal effects and includes the specific locus method.

Mutation induction by seed treatment.—Barley (*Hordeum*) has been used extensively in the study of induced hereditary changes. This plant can be recommended because of the extensive knowledge of its genetics including numerous and distinct chlorophyll-deficient mutations (*76*) and because of the low number ($2n=14$) of relatively large chromosomes. The seed is very easy to store, treat and handle, and the seedlings are small and easy to grow. Responses to mutagen treatment which may be measured include: (1) Chromosome aberrations in shoot- or root-tip cells of treated seeds; (2) chromosome aberrations in the pollen-mother-cells of M_1 plants; (3) chlorophyll-deficient mutations; (4) pollen abortion; (5) alteration of the observed mutation spectrum of M_2 seedlings; (6) seedling growth reduction; (7) survival; (8) spike fertility; and (9) yield of spikes *per* plant.

The complete techniques for handling mutagen-treated barley seeds (*54*), as well as more detailed descriptions of both laboratory (*70*) and field culture of seedlings (*71*) have been described. These techniques are easily modified for use with seeds of many other higher plant species.

The basic difficulty with progeny testing for mutation in higher plants is the long generation time involved. By growing the M_2 generation in the greenhouse, the time has been reduced to less than 1 year with barley. However, this is still too long for rapid screening of mutagens. The techniques with barley have been developed to the point that it is known that there is excellent correlation between the M_1 seedling growth inhibition, and the M_2 seedling chlorophyll mutation frequency (*77*). This relationship also exists between seedling growth inhibition and chromosome aberrations in the M_1 shoot or root tip (*10*). Therefore, in 1 week rapid data may be obtained concerning the mutagenicity of a compound. Another possibility in seed mutagenesis is to use small more rapidly growing plants such as *Arabidopsis* as has been done with much success by Rédei and Li (*84*). This plant is sensitive enough to detect low frequencies of mutation induced by DNA base analogs (*38*).

A limitation of the above method, namely progeny testing, is overcome if seeds, heterozygous for a marker gene, are used. This specific-locus technique was employed by Smith (*93*) on the Yg_2/yg_2 (light-green) locus in maize (*Zea mays*). The yg_2 is nonlethal in homozygous condition and the Yg_2/yg_2 seeds are produced by crossing Yg_2/yg_2 to female yg_2/yg_2. This same technique could be employed in barley, where efficient methods for production of hybrid seeds now are available (*82*). *Arabidopsis* gives the combined advantages of somatic detection, a short generation time and small size (*65, 84, 94*).

Root tip method for chromosome aberrations.—Root tips of certain plant species provide excellent material for chromosome aberration studies and have been extensively used for this purpose following exposure to chemical mutagens (*61*). Appropriate species are easy to obtain and grow, easy to treat with aqueous solutions, and have several large root tips providing a large cell population. Also, many have a relatively small number of large chromosomes and hence analysis of the numbers and kinds of aberrations produced is relatively easy. Treatment periods are short (minutes to hours) but fixations should be made up to 48 hours.

At the present time, chromosome scoring is done by eye but the small number of large chromosomes should make plant material excellent for computer assisted analysis. Recommended material with diploid chromosome numbers are: *Allium cepa* (*16*) *Bellevalia romana* (*8*), *Campelia zanonia* (*16*), *Crepis capillaris* (*6*), *Haplopappus gracilis* (*4*), *Hordeum* (*14*), *Lilium* (*24*), *Tradescantia* (*12*) and *Vicia faba* (*12*). Suitable cytological methods are described in various publications, *eg.*, Darling and LaCour (*15*) and Sharma and Sharma (*91*). The method can be developed for fairly rapid screening.

Somatic Mutation Methods.—*Tradescantia* plants heterozygous for flower color provide a useful test system for physical or chemical mutagens. This plant is relatively easy to grow under a wide range of environmental conditions, blooms continuously throughout the year thus providing material for somatic mutation analysis, has 12 large chromosomes and, coincidentally, has a cellular radiosensitivity similar to that of mammalian cells. Special clones, heterozygous for flower color, can be used for easy detection of somatic mutations in both petal and stamen hair tissues using only a dissecting microscope and elementary laboratory techniques (*42, 67, 72, 97*). These clones can be readily propagated by cutting, and root easily to provide material for chromosome analysis (*99*). Chromosome aberrations can also be studied in various other tissues (petal, stamen hair (*17*), and during microsporogenesis (*25*)).

Young flower buds on intact plants or on cuttings may be exposed to various mutagens in either a gaseous or aqueous state. Material for cytological studies may be fixed within 24 hours after treatment; pollen abortion in mature flowers may be observed with peaks at 5 to 7 and 16 to 20 days after treatment, reflecting injury induced during microspore mitotic and meiotic stages respectively; loss of reproductive integrity of stamen hairs reaches a maximum at about 14 days; and somatic mutations and morphological changes in petals and stamen hairs may be scored throughout a 10- to 20-day post-treatment period; stamen hairs (*17*) and haploid pollen tubes (*95*) provide excellent material for chromosome analysis.

Various other genera and species have also been used to detect somatic mutation and morphological changes in petals and stamen and should be equally useful in chemical mutagen studies (table 1).

Specific-locus method (waxy locus) in pollen.—The *waxy* locus in maize, barley, and rice determines the type of starch which is synthesized in the triploid endosperm and in the haploid pollen grain. In the case of the pollen grain the phenotype is determined by its own genotype and not by the genotype of the plant. The dominant *wx* pollen grains stain blue with an iodine-potassium iodide stain while recessive *wx* pollen grains stain a reddish-brown color (*73*, *74*) because *wx* pollen lacks the enzyme required in the last step of starch formation. Since the wild type is *wx*, the frequency of induction of *wx* can be assayed in millions of pollen grains relatively easily and quickly. Furthermore, the phenotype appears in the treated generation, and does not require the time necessary to obtain an M_2 generation. This technique was used in barley by Eriksson (*23*) who irradiated plants homozygous for *wx* and analyzed the frequencies of reversions from the *waxy* phenotype to the wild type, and Baldi, (*3*) who studied the spontaneous back mutation rate at this locus in rice. The frequency of intra-cistron recombination may also be measured with this technique by crossing two *wx* mutants of independent origin and collecting and staining pollen from the F_1 as done by Briggs and coworkers (*6*, *7*) for EMS- and radiation-induced mutations. This is a very simple and rapid technique for detecting even very low frequencies of induced mutations in higher plants.

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TABLE 1.—*Summary of experimental procedures for detecting various effects of chemical mutagens using certain higher-plant test systems.*

Stage treated	Analyses used for detecting various effects								Species used and literature reference
	Somatic cells		Germinal cells		Microspores or Pollen tubes	Pollen			
						Mutation		Chr. Ab.[3]	
	Mut.	Chr. Ab.	Mut.	Chr. Ab.	Chr. Ab.	Phenotype[4]	Lethal	Translocation	
Seed or seedling	(2)	(2)	P[3]	(2)	(2) (5)	(2)	------	(2)	*Allium*[59, 90] *Arabidopsis*[38, 65, 84], *Avena*[39], *Hordeum*[21, 22, 55, 57, 76, 108, 111], *Oryza*[81, 112], *Triticum*[56], *Zea*.[93]
Flowering:									
Somatic Cells [1]	(2)	(2)	------	------	------	------	------	------	*Antirrhinum*[98], *Cosmos*[31], *Dianthus*[13], *Gladiolus*[97], *Haemanthus*[69], *Lilium*[98], *Petunia*[98], *Tradescantia*[17, 40, 41, 42, 66, 72, 97], *Tulipa*.[97]
Meiosis	------	------	P	(2)	(2)	(2)	(2)	------	*Hordeum*[23], *Tradescantia*[33] *89*, *Trillium*[101], *Tulipa*[75], *Vicia*[64], *Zea*.[24]
Microspore	------	------	------	------	(2)	------	------	(3) (5)	*Campelia*[88], *Tradescantia*[4, 25, 26, 89, 100, 105].
Pollen	------	------	P	------	(2)	------	------	------	*Hordeum*[62], *Lilium*[5], *Petunia*[5], *Tradescantia*[5, 95] *Zea*[5].

---

[1] Mostly petals and stamen hairs.

[2] Translocations produce pollen abortion.

[3] P=progeny testing.

[4] Especially waxy locus in barley, maize and rice.

[5] Micronuclei can be counted as a fast screening method.

Table 2.—*Partial list of chemicals known to produce mutations or chromosome aberrations in higher plants with literature citation.*

Chemical	Mutation	Chr. Aber.
Acridine (and derivatives)		12
Alkaloids:		
Colchicine	85	
Morphine		80
Scopalamine		80
Amines, and related compounds:		
Acetylethyleneimine		79
2-chlorotriethyl amine	57	
Ethyleneimine	22	
Hydrazine	43	
Hydroxyurea		49
Maleic hydrazide	29	16, 29
N-methylphenylnitrosoamine		48
Nitrosoamines	107	
Triethylene melamine		8
Antibiotics, and related compounds:		
Aminopterin	65	47
Streptonigrin		51
Nebularine (9-β-D-ribofuranosylpurine)	110	
Bromine		9
Ceepryn		96
2,2-dichlorovinyl dimethylphosphate (Vapona)		90
Diethyl sulfate	34, 57	
Epoxides:		
Diepoxybutane	21	
Ethylene oxide	34, 103, 110	95
Glycidol	19	
Food additives:		
Butylated hydroxy toluene		90
Butylated hydroxyanisole		90
Coumarin		11
Sucaryl		90
Hexachlorocyclohexane		58
Isopropylphenyl carbamate		18
Mercury compounds:		
Ethyl mercuric phosphate		87
Methyl mercuric hydroxide		83
Phenyl mercuric hydroxide	63	63, 83
Mustards:		
Sulfur mustard		14
Nitrogen mustard	37, 60	14, 78
Nucleosides:		
adenine arabinoside (arabinosyl-adenine)		52
adenine xyloside (xylosyl-adenine)		52
5-bromodeoxycytidine	38	
5-bromodeoxyuridine	38	
deoxyadenosine		47

Table 2.—*Partial list of chemicals known to produce mutations or chromosome aberrations in higher plants with literature citation*—Con.

Chemical	Mutation	Chr. Aber.
Nucleosides—Continued		
cytosine arabinoside		47
5-fluorodeoxyuridine (FUdR)		47, 106
1-methyl-3-nitro-1-nitroso-guanidine		44
N-methyl-N′-nitrosoguanidine	92	
Pesticides: (See table III.)		
β-propiolactone		96, 104
Purines:		
2-aminopurine	32, 53	
caffeine (1,3,7-trimethyl-xanthine)		50
8-ethoxycaffeine		45, 51
1,3,7,9-tetramethyluric acid		51
Sulfonic acids and derivatives:		
methane sulfonate-bromoethyl	36	
n-butyl	57, 77	
chloroethyl	36	
ethyl	1, 2, 6, 84	
β-hydroxyethyl	28	
β-methoxyethyl	28	
methyl	57, 68	
isopropyl	57, 77	
n-propyl	20, 57	
β-methane sulfonyl oxybutane (Myleran)	32, 36, 110	110
diethyl 1,3-propanedisulfonate	110	
o-sulfobenzoicimide (Saccharin)		90
Urethanes:		
ethyl		80
N-nitroso-N-methyl		48

Table 3.—List of various pesticides (1,000 p.p.m., 12 hrs.) known to produce mutations in barley and relative efficiency of each to control and to 5,500 R of X rays (Wuu and Grant, *111*)

Treatment	Relative efficiency	Treatment	Relative efficiency
Lorox	30	Botran	7
Simazine	24	Phosphamidon	7
ENT-50612	14	Alanap-3	4
Atrazine	10	Metepa	4
Monuron	10	Endrin	3
Embutox E	9		
Sevin	9	X rays	32
Banvel D	7	Control	1

Table 4.—*Maximum percent mutations reported for a series of mutagens tested on several organisms* (*57*)

Agents	Mutations at several loci		Mutations at specific loci	
	Barley (*Hordeum*) Chlorophyll mutants mutated spikes [1]	Drosophila sex-linked rec. lethals [2]	*Neurospora* *ad* reversions [3]	*Schizo. pombe* *arg* reversions [4]
Gamma rays	17			
Diethyl sulfate (dES)	43		18	0·1200
Methyl methanesulfonate (MMS)	33	11·6		0·0220
Ethyl methanesulfonate (EMS)	57	39·0	17	0·0910
Chloroethyl methanesulfonate			51	9·9220
n-Propyl methanesulfonate (nPMS)	26			
Isopropyl methanesulfonate (isoPMS)	20			
n-Butyl methanesulfonate (nBMS)	28	·8		
Ethyl ethanesulfonate (EES)	25			
Nitrogen mustards:				
2-chlorotriethylamine	15			0·0009
chlorodimethylamine			1·7	
Ethyleneimine	28		16	
Diepoxybutane			85	0·0160
Glycidol	22		34	0·0240
Ethylene oxide	13		17	

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[1] After compilation by NILAN et al. (*76, 77*).
[2] Data from FAHMY and FAHMY (*27*).
[3] After compilation by WESTERGAARD (*109*).
[4] Data from HESLOT (*35*).

Drosophila.—While there are a considerable number of mutation tests that are capable of being carried out on Drosophila (*1*), we refer here primarily to those which in general are simple, rapid and unambiguous in interpretation. They are also reasonably inexpensive to perform. The types of tests described below may be run independently, or two or more tests may be carried out on the same group of treated individuals by using special stocks. Detailed procedures will not be presented; however, many of the tests are discussed in general genetics texts or in references listed below (*2*).

Probably the most widely used experimental procedure is the sex-linked recessive lethal test. Either sex may be treated and mutation frequencies from successive germ cell stages may be obtained. The test requires that two generations be bred; however, chemical mutagens often produce delayed or mosaic effects and a third generation may be necessary. Large numbers of progeny may be tested and since a lethal is indicated by the absence of an entire class of flies, the test is objective. Lethals are among the most commonly induced mutations. While the number of gametes analyzable will vary with the number of persons employed, a staff of 2 or 3 can screen between 5,000 and 10,000 X-chromosomes a month.

The purpose of the experiment with mutagens should determine the experimental precautions employed. Whenever quantitative mutation frequencies are required in order to compare, for example, results from different mutagens or different cell stages etc., then the age of the flies, the breeding periods, the cell sampling procedures as well as other physiological and environmental variables must be rigorously controlled. On the other hand, if only a relative index of mutagenicity is sought, these variables need not be as stringently controlled.

The two generation reciprocal translocation test is one that is in general use in many laboratories. The test is similar to the sex-linked lethal test, requiring single cultures for each F_1 individual tested. Screening of F_2 progeny is more difficult and time consuming than for lethals, but the test is an objective and reliable index of chromosome breakage. Meiotic and post-meiotic male germ cells are most effectively studied. Four to six weeks (if retests are carried out) may be required to complete the translocation test.

Sex chromosome loss experiments are a one-generation test which detect either complete or partial loss of the sex chromosomes, the loss resulting primarily from chromosome breakage. The test is useful because either sex may be treated, the phenotypes of the exceptional classes of offspring are readily discernible from the normal progeny, and large numbers of flies may be rapidly screened with each individual representing a treated gamete. It should be possible to examine a minimum of 5,000 progeny from treated gametes *per* day *per* investigator. Although many more chromosomes can be tested *per* man hour by this method than by the recessive lethal method, many mutagens may be more effective at inducing lethals and other point mutations than chromosomal loss.

A second one-generation test of great usefulness in detecting chromosome rearrangements induced in either sex is the bithorax method of Lewis (*3*). A conspicuous enhancement of the bithorax phenotype signals a chromosome rearrangement, translocation or inversion, involving chromosome 3 (one of the two large autosome pairs of *Drosophila*). Each F_1 represents a treated gamete and only the exceptional progeny need be further analysed to verify the transmission and to determine the nature of the change. Probably no more than

4,000–8,000 chromosomes can be analysed *per* week by a single worker. Spontaneous rearrangements are extremely rare.

A third chromosome breakage study applicable only to oocyte testing involves detachment of attached X-chromosomes (4, 5). A simple phenotypic difference permits rapid classification of the normal from the exceptional progeny. The spontaneous frequency of detachment is of the order of 1 *per* 1,500 gametes. Probably 10,000–15,000 chromosomes from an array of oocyte stages can be tested *per* week *per* person. The mature oocyte is perhaps the stage of greatest sensitivity to chromosome damage as indicated by irradiation studies.

All of the last three mentioned tests need not be counted "by hand". The investigator can screen for the exceptional flies and the rest can be counted rapidly and accurately by an electronic fly counter (*6*).

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Mammalian methods

Cytogenetics and somatic cell genetics.—In appraising any method for mutagenicity testing, it is important to be clear as to what we are asking of the method. After this, the advantages and disadvantages of any test system can be better assessed. With a cytogenetic test system, we are seeking for morphological evidence of damage to the genetic material. With this in mind, some of the obvious advantages are the wide number of species, including human, that can be examined by these methodologies; the fact that it can be performed on both *in vivo* and *in vitro* systems; the genetic material is being observed directly, and the tests can be accomplished relatively rapidly with limited expense. Disadvantages include the fact that it needs a well-trained examiner for accurate results; there are possibilities of subjective errors; procedures need to be standardized, at least within certain limits, in order to have the tests reproducible from laboratory to laboratory; and there isn't complete agreement on definitions and classification of breaks and gaps and the various abnormalities. However, the primary disadvantage is that there is no proof that seeing cytogenetic abnormalities is an absolute indication of mutation. One can visualize a spectrum of damage from such severe damage that the cells die without ever getting into mitosis, to other cells that are made incapable of cell division but survive in a post-mitotic state, with or without a change in functional proteins; and finally, gene mutations, in which cells can still go on to divide but have alterations in the functional proteins produced.

Evidence for the first two types of change seems well established, and this is of course important in our consideration of damage to genetic material, and is a very important consideration in teratogenesis and perhaps in aging. The mutations are less easily confirmed, however. If chromosome breakage is important in mutation, it would express the view that the breaks are an indicator system, since most of the cells with visible unstable chromosome abnormalities would probably go on to cell death. Work correlating mutation and chromosome breakage after chemical treatments is in an early stage when compared to the data available for X-rays. However, Kihlman has pointed out that there is good correlation between substances that are capable of producing mutations in various systems and those that can produce chromosome abnormalities (*3*). There is almost 100 percent correlation between chromosome aberrations produced in mammalian cells in tissue culture and mutagenic effects whenever there was data available on both effects (see table 5) (*3*). The correlation between mutagenesis and chromosome aberrations in plant root tips was good, but not as good as for mammalian cells in tissue culture.

The absolute answer to this question of chromosome breaks serving as an indicator for gene mutation will probably come from the studies that are presently starting in somatic cell genetics when these are correlated with, and studied in conjunction with, cytogenetics. The type of work referred to here is the ability of an agent to induce drug resistance in somatic cells which reflects the loss of functional enzyme, as for example resistance to BUdR, because it is no longer incorporated into the cell due to the loss or modification of the thymidine kinase enzyme. This approach is well exemplified in recent studies in which Chinese hamster cells were treated with BUdR and nutritionally deficient mutants were selected by growing cells in restrictive media in which the nutritionally deficient mutants cannot divide, and then adding an agent that will kill dividing cells (*5*). Similarly, selective culture techniques have been used to isolate L glutamine auxotrophs or 8-azaguanine resistant Chinese hamster cells and to compare the incidence of these mutants in cultures treated with chemical mutagens and control cultures (*1*). Human male cells and genes on the X-chromosome have been similarly studied (*2*). Loss or deficiency of the enzyme hypoxanthine-guanine phosphoribosyl transferase (HG–PRT) imparts resistance to purine analogues. Since the gene for this enzyme is located on the X-chromosome, the use of male cells permits detection of changes in the single gene. Also, somatic cell genetic systems utilizing isoenzymes *via* their electrophoretic patterns, offer an excellent definitive tool. Changes in these isoenzyme patterns can be proof of mutation in the cultured cells. Besides confirming the relation of chromosome breakage to mutation, these somatic cell genetic systems should provide an excellent methodology for mutagenicity testing in their own right, as they are further developed.

The importance of demonstrating whether or not breakage is an indicator for mutations lies in the areas of carcinogenesis, germline mutation with increasing genetic load of the population, and perhaps in some aspects of aging.

Concerning methodology itself, preparations can be made very rapidly from tissue cultures for *in vitro* preparations that have the advantage of short time of experiments; such preparations additionally are usually morphologically better than *in vivo* preparations. Readily available cultures from *Potorous*, designated PTK–1, are exceedingly well suited for cytogenetic studies in that chromosomes are large, distinct, and there are only 11 in number. The Chinese hamster, with 22 chromosomes, has many of the same advantages, and of course there are both human leukocyte cultures and diploid human fibroblast cultures that have the advantage of being from the human species. The leukocyte cultures have the additional advantage that cell cycle is not initiated until phytohemagglutinin is added, so that timing for adding various agents for various portions of the cell cycle can be done with greater precision than in many culture systems.

The *in vivo* assays offer many of the same advantages of the host-mediated assay utilizing bacteria. That is, breakdown products and other metabolic products of the test agent have a chance to produce effects as well as the agent itself. Bone marrow, spleen, and testes are especially suitable for *in vivo* preparations, as well as embryo homogenates and tissues.

From all of these materials, both metaphase and anaphase preparations can be made. Metaphase has the advantage of excellent morphologic detail of each chromosome so that localization to specific chromosomal areas can be accomplished. Anaphase has the advantage that the pretreatment is much reduced, and the rapidity with which anaphase preparations can be read is much greater, and the experience necessary to become competent in anaphase evaluation is considerably less than for a similar degree of competence with metaphase.

Classification of chromosomal defects is not standardized at the present time. Various factors are used in different classification methods, and a brief appendix of some of the types of classifications is included.

Some difficulties that have arisen in the past, as a differentiation between gaps and open breaks, or the degree of significance of open breaks vs. rearrangements, would seem on the basis of present information not to be as big a problem as was once imagined. Gaps and breaks both seem to increase in parallel in most of the systems studied up to now, so any method of differentiation between the two, as long as it is standardized, is adequate to compare control with experimental material, even though it is arbitrary. Similarly, the difference between open breaks and chromosome rearrangements would appear to be whether or not cellular DNA and/or protein synthesis is inhibited, or can continue. In the absence of DNA and/or protein synthesis, healing is inhibited, and it is the healing that permits rearrangement. Many of the materials that have been shown to produce only open breaks in acute studies, are seen to progress to chromosomal rearrangements when chronic studies are carried out allowing a recovery period. This difference stresses a need to carry out a portion of the studies in a cytogenetic test system after the test substance has been removed and a recovery period allowed.

In summary, cytogenetic studies would certainly seem to be one of the best screening methods for testing of mutagenicity, but should be used in conjunction with other additional methods. Cytogenetic testing reveals a variety of damage to the genetic material in addition to mutation, as well as a high correlation with mutagenic events when both parameters are tested. It offers *in vivo* and *in vitro* methods for a wide variety of species including the human. When cytogenetic studies are a method employed for screening, standardization of procedures, high quality of preparation, and reading of coded slides are essential for best results.

Classification of chromosome breakage.—Unfortunately, there is no single classification of breakage, since different characteristics of breaks have been used by various authors to classify them. This leads to some confusion and redundancy, but in general, the various classifications are consistent, one with the other. The characteristic that has been used to classify has frequently depended on the type of study under way and the information sought.

One of the main characteristics used to classify chromosome breaks has been whether one or both of the two chromatids of the chromosome are involved in the defect. If both chromatids are involved, the defect is called a chromosome break, while if one chromatid is involved it is

TABLE 5.—*Comparison between chromosome-breaking and mutagenic effects of chemicals in plant and animal materials.*

Compound	Chromosomal aberrations		Mutagenic effect
	Plant root-tips	Mammalian cells in tissue culture	
Adenine	+	+	+
2,6-Diaminopurine	−	+	+
Caffeine	+	±	+
8-Ethoxycaffeine	+	±	±
Purine riboside	−	+	+
Deoxyadenosine	+	+	No data
5-Fluorodeoxyuridine	+	+	No data
5-Bromodeoxyuridine	−	+	+
Cytosine arabinoside	−	+	No data
Maleic hydrazide	+	−	−
Azaserine	+	+	+
Streptonigrin	+	+	+
Mitomycin C	+	+	+
Hydroxylamine	±	+	+
Nitrogen mustard	+	+	+
Triethylenemelamine	+	+	+
Diepoxybutane	+	+	+

+ marked effect.
− no effect.
± effect very low, although just about significant.

From Actions of Chemicals on Dividing Cells, B. A. Kihlman, Prentice Hall, 1966, pp. 198.

Comparison between the Effects of Chemicals on Animal and Plant Cells.

termed a chromatid break. The factor that determines which type of lesion is produced is whether or not the chromosome is a single unit or a double unit at the time of the insult that produces the break. This in turn is dependent upon the stage of the cell cycle. If a chromosome is in the GI phase of the cycle before DNA synthesis has taken place, it is a single structure, and if a break is produced at this time, the break is replicated along with the second chromatid during the S or DNA synthesis period resulting in a chromosome break. If the breaking insult occurs during G2 or thereafter, after DNA synthesis, when the chromosome is already a dual structure, then a chromatid break is the usual result. During the period of DNA synthesis, a combination of both types of breakage can be found in the same cell, depending on whether the individual chromosome had not yet started, or had finished synthesizing its DNA. It does occasionally happen that an event affects both chromatids after they are a double structure, and in this case the term "isochromatid break" is used, indicating that the lesion was one introduced in chromatids, but that both chromatids were affected at the same point. This is distinguished from chromosome breaks only by the fact that other lesions in the same material are predominantly chromatid breaks.

An additional type of breakage using these criteria has been described by Ostergren and Wakonig and termed a "delayed isolocus break". These authors described a typical example of this kind of breakage as a secondary constriction in one chromatid with a corresponding break in the isolocus position in the other. In addition to this typical lesion, other chromosomes would exhibit everything from only a secondary constriction in one chromatid to a complete break in both chromatids. At the time of the description of this type of breakage, the authors felt that a partial defect was produced in the chromosome when it was a single unit, and then this partial defect was reproduced in both chromatids at the isolocus point during DNA synthesis. Mitotic forces and pressures subsequent to this were thought to produce the variety of possible changes at the isolocus spots in the chromatids.

An alternative explanation would be that this type of breakage occurred during the period of DNA synthesis and affected different chromosomes differently, depending on the state of synthesis of that particular chromosome.

A second important characteristic that has been used in classification of chromosome breaks is dependent on whether or not healing or reunion has occurred. If there is no healing, an open break or defect is the result, and this has also been termed a "simple break" and a "terminal deletion". In this type of breakage, a significant problem arises in distinguishing between a break which is defined as a "complete discontinuity" between the two chromosome pieces, and a "gap", which is defined as an achromatic or unstained area in which chromatin still exists but is difficult to see. Various methods have been used to make this distinction. Some authors insist on displacement of the distal fragment before considering it a break, while others, have established an arbitrary distance between the two stained chromosome pieces as the distinguishing factor. We recommend that any defect separated by at least the width of one chromatid be regarded as a break, and anything less than this as a gap. This is admittedly arbitrary, and undoubtedly frequently incorrect, but serves as a basis of comparison between experimental material and control material. It is fortunate that in most systems, gaps and breaks seem to increase and decrease in parallel, so that the methods such as described although arbitrary and inaccurate in the literal sense, enable valid comparisons between various test materials.

When healing or reunion occurs, it is possible for restitution to occur if the broken ends reunite in their original positions. In this case no defect is visible. If they do not heal in their original positions, then a structural rearrangement is the result. These are often further divided into an intra-change if the rearrangement is within a single chromosome, or an inter-change if the rearrangement involves more than one chromosome. Both of these can be further divided into symmetrical and asymmetrical defects. A symmetrical defect is one in which no mechanical difficulty results during mitosis, and either daughter cell is deficient in chromatin material. An asymmetrical intra- or inter-change is one in which either mechanical defects arise or the resulting daughter cells are deficient in chromatin material (*4*).

Another term that is frequently used in a very similar context with symmetrical and asymmetrical is stable and unstable rearrangement. The primary factors that determine whether the open or simple type of breakage will result, or the rearrangement will result, seems to be whether the cell retains the ability to synthesize protein and/or DNA. If either or both of these processes are interrupted, there is evidence that reunion cannot take place and open breaks result.

In addition to these classifications used when the cells under study are examined in metaphase, which affords greater morphologic detail of individual chromosomes due to various pretreatments including colchicine, hypotonic expansion, and air drying or squashing, it is also possible to score defects in anaphase preparation. Here, none of the previously mentioned pretreatments are used and the cells are merely fixed and stained. The types of anaphase aberration that can be distinguished include an acentric fragment, which is a paired segment of chromatids left at the equator of the cell resulting from a chromosome break; an attached fragment in which a chromatid fragment is away from the main body of anaphase chromosomes, but is oriented in line with the chromosomes and seems to be attached by an attenuated portion; a chromosome bridge which results from an asymmetrical rearrangement as a dicentric chromosome or an interlocking ring chromosome; finally, pseudochiasmata, which are thought to result from two chromosomes adhering to each other *via* stickiness or some other mechanism, and may very likely not represent true defects.

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The host-mediated assay.[1]—A great deal of recent work in genetics has served to point out the universal nature of the genetic code. Although the level of organization of genetic material in bacteria is different from that in man, there is no basis for assuming that the action of a mutagen will be markedly different. It is essential, however, to properly define the ultimate mutagenic agent occurring in the mammalian host. There are numerous examples of compounds that are not mutagenic in micro-organisms, but are converted to active mutagens in animals, and there are many compounds that are active in micro-organisms but detoxified in mammalian systems. The host-mediated assay was developed to determine the ability of laboratory animals to either activate or detoxify compounds in regard to their mutagenic activity.

In this assay, the mammal, during treatment with a potential chemical mutagen, is injected with an indicator micro-organism in which mutation frequencies can be measured. It is important to note that mutagen and organism are administered by different routes. After a sufficient time period, the micro-organisms are withdrawn from the animal and the induction of mutants is determined. The comparison between the mutagenic action of the compound on the micro-organism directly and in the host-mediated assay indicates whether the host can modify the compound and whether mutagenic products can be formed as a result of host metabolism. The formation of mutagenic metabolic products from dimethylnitrosamine, and the plant toxin, cycasin, have been reported using this procedure.

Indicator micro-organisms presently being used in this procedure include the histidine auxotroph of *Salmonella typhimurium*, and *Neurospora crassa*, where scoring for forward mutations is carried out. In the *Salmonella* system, a number of known auxotrophs are injected intraperitoneally in an animal previously treated with the chemical. After six generations, approximately 3 hours, the organisms are recovered from the intraperitoneal cavity and the induction of mutation determined. The effect in the animal is compared with the effect of the chemical in an *in vitro* plate assay. In the *Neurospora* system, *conidia* from the *Neurospora dikaryon* described earlier can be injected into the peritoneal cavity or subcutaneously in mice or rats. In rats, the *conidia* can also be injected into the testis. After 48 hours, 50–70 percent of the *conidia* can be recovered with approximately

50–60 percent viability. Since the *conidia* can be kept in the animal for an extended period of time, it is possible to do meaningful feeding experiments in which one can test for presence of mutagenic compounds in the diet. After the *conidia* are recovered, they are tested for presence of spontaneous and induced ad-3 mutations. A more ideal indicator utilizing a forward mutation system in bacteria is presently being developed. It is probable that the newly developed methodology for scoring forward and reverse mutations in cultured cells might also be adopted in this procedure.

In addition to flexibility in selection of indicator organism, almost any laboratory animal can be used. Laboratory animals including rats, mice, and hamsters have been successfully utilized. Not only can we compare mutagenic activity between micro-organisms and mammals, but also between different animal species. It should also be possible to demonstrate any correlation between mutagenicity and carcinogenicity in the same or different animals.

The host-mediated assay is an ongoing procedure that bridges the gap between simple microbial tests and the effects of a potential mutagen in mammals. Similarity between mutagenic activity in micro-organisms and animals, the ability of the mammal to detoxify mutagenic or nonmutagenic agents, and the production of mutagenic metabolites can be determined. Not only can comparisons be made between micro-organisms and mammals, but also between different animal species. It is quite possible to compare mutagenicity and carcinogenicity in the same system with this procedure. However, the host-mediated assay in no way indicates the effect of DNA repair mechanisms of the host in response to specific chemicals, and is only an indirect measure of mutagenicity in terms of the mammalian host.

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Specific locus test. (*1*, *2*)—The specific locus test is based on detection of newly induced mutation in seven coat-color and morphologic loci in mice. The newly induced mutations can either be chromosome deletions or point mutations. In this test, male mice that are homozygous for the dominant trait are given the suspected mutagen and mated with female mice that are homozygous for the recessive traits. In this way, the occurrence of offspring with recessive characteristics is indicative of mutation or loss in the gene.

The following potent mutagenic compounds: methyl methanesulfonate, ethylmethanesulfonate, propylmethanesulfonate, and isopropyl methanesulfonate, were tested in both the dominan lethal and in specific locus tests. All four mutagens were highly positive in the dominant lethal test, but only slightly positive or negative in the specific locus test. However, the results are difficult to compare because sperm which were used in the two tests derived from cells which were treated at very different stages of their development.

The number of animals which has to be used to detect a doubling of mutation frequency is so great that the expense of this test makes it quite impractical to use as a general screening technique. More seriously, however, is the failure to detect a significant increase in mutations with the above four strong mutagens, either because of lack in statistical power or because of intrinsic defects in the test system, strongly militates against the practical utility of the specific locus test.

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Dominant lethal test.—Dominant lethal mutants are convenient indicators of major genetic damage which have been used in mammals for measuring effects of X-rays (*1*), and more recently, of chemical mutagens (*2*, *3*, *7*, *8*, *13*, *15*, *22*). Data on induction of dominant lethal mutants in mammals may be appropriately extrapolated to man, especially as most recognizable human mutations are due to dominant autosomal traits (*21*). The genetic basis for dominant lethality is the induction of chromosomal damage and rearrangements, such as translocations, resulting in nonviable zygotes; evidence for zygote lethality induced in mammals by X-rays and by chemical mutagens has been obtained embryologically (*16*, *25*, *26*), and cytogenetically (*4*, *15*, *17*, *23*), respectively. Additional evidence for the genetic basis of dominant lethality is derived from the associated induction of sterility and heritable semisterility in F_1 progeny of males exposed to X-irradiation (*19*, *25*) and to chemical mutagens (*5*, *12*, *14*); translocations have been cytologically demonstrated in such semisterile lines in mice (*7*, *18*, *24*), and in hamsters (*20*).

The induction of dominant lethal mutations in animals can be assayed, with a high degree of sensitivity and practicality, following acute, subacute or chronic administration of test materials, either orally or by any parenteral route, including the respiratory. For these reasons it is feasible to integrate such tests in the scope of routine toxicological practice (*9*). Following drug administration to male rodents, they are mated sequentially with groups of untreated females over the duration of the spermatogenic cycle. For mice, the entire duration of spermatogenesis is approximately 42 days comprising the following stages: spermatogonial mitoses—6 days, spermatocytes—14 days, spermatids—9 days, testicular sperm—5.5 days, and epididymal sperm—7.5 days (*1*). Thus, matings within 3 weeks after single drug administration represent samplings of sperm exposed during postmeiotic stages, and matings from 4–8 weeks later represent samplings of sperm exposed during premeiotic and stem cells stages.

The classical form of the dominant lethal assay involves autopsy of females aproximately 13 days following timed matings, as determined by vaginal plugs in mice and vaginal cytology in rats, and enumeration of corpora lutea and total implants, as comprised by living fetuses, late fetal deaths, and early fetal deaths. The test can be considerably modified and simplified and hence made more suitable for routine practice by sacrificing the females at a fixed time, *e.g.*, 13 days in mice, following the midweek of their caging and presumptive mating. Additionally, this allows determination of effects of drugs on pregnancy rates. Similarly, corpora lutea counts, which are notoriously difficult, laborious, and inaccurate in mice and afford a measure of total fertilized zygotes, can be omitted and numbers of total implants in test animals can be related to those in controls, thus affording a simple measure of preimplantation losses. Using such modified procedures together with computerized data handling, large numbers of test agents can be simply and rapidly tested for mutagenic activity. The assay can also be conducted with drug administration to female mice, either before or in early pregnancy; however, this test has not yet been developed for routine purposes.

Dominant lethal mutations are directly measured by enumeration of early fetal deaths, and indirectly by preimplantation losses, as measured by reduction in the number of total implants in test compared with control females. Results are best expressed as early fetal deaths *per* pregnant female, rather than as the more conventional mutagenic index, early fetal deaths x 100 *per* total implants, as the latter index can be markedly altered by variation in the number of total implants (*11*). Preimplantation losses offer a presumptive index of mutagenic effects, but there is no precise parallelism between preimplantation losses and early fetal deaths. These should be regarded as concomitant and not alternate parameters. Furthermore, the use of the mutagenic index presupposes that the number of early deaths is proportional to the number of implants regardless of preimplantation losses; this would anticipate that absolute number of early deaths are lower in those animals with reduced numbers of total implants. This has been shown experimentally not to be so (*11*). Finally, an additional disadvantage of such ratios as measures of mutagenic effect is that their variability is high, as both numerator and denominator are contributory, and estimates of standard deviation, hence, are complex.

Preimplantation losses, early fetal deaths, sterility and semisterility constitute a spectrum of adverse genetic effects, of which early fetal deaths clearly afford the most convenient and quantitatively unequivocal parameter of mutagenicity.

Using these techniques, a wide range of chemicals to which man is exposed in the totality of the environment, including pesticides, food additives, drugs, air and water pollutants, have been tested for mutagenicity in mice (*10*, *13*). Additionally, detailed dose-response studies with the aziridine alkylating agents, TEPA and METEPA, which have been used as chemosterilant pesticides, have revealed mutagenic thresholds in the region of 0.04 mg./kg. and 1.4 mg./kg., respectively, following acute single parenteral administration in mice.

These techniques are also ideally suited for the study of synergistic or antagonistic effects on mutagenesis; caffeine, for example, has been shown not to induce dominant lethal mutations nor to synergize the mutagenic effects of X-rays or of alkylating agents (*10*).

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Population monitoring. Whatever the system of testing potential pesticides before they are used may be, it can never be perfect. There is always the possibility that some substance will not be revealed as mutagenic by any of the test systems employed and yet represent a mutation risk to man. An example might be a substance that is not itself mutagenic, but which is specifically converted by the human body into a substance that is strongly mutagenic. If such a compound was widely used, we could be doing great harm to our descendants and never discover this fact until the damage had already occurred. And genetic damage, as we have emphasized, is irreversible as far as is now known.

Is there any possibility of setting up a system to detect such a genetic emergency if it should occur? The task would be enormously difficult, for many reasons already mentioned. For one thing, the damage caused by mutations occurs in future general generations, not this one, so the effect would not be observed for some time. In the second place, the effect might be spread out over many generations so that an enormous total effect would still be small enough in the first generation not to be noticed. Finally, the kinds of effects produced by mutations are not unique, so if there were, for example, an increased disease or death rate it would be very difficult to be sure that this were due to mutation and not some other cause.

We have to accept for the present the fact that any feasible system of monitoring the human population could detect only a very gross effect. But, of course, that is what is the most to be feared. So there may be merit in setting up a system that would detect at the earliest possible date a really large increase in the mutation rate—say an increase of several fold—if this is occurring. Could such a system be made workable, and not prohibitively expensive? We don't have the answers now, but we would like to suggest a few possibilities which might merit further consideration. The problem is larger than just pesticides, and would have to be considered in a wider context of detecting any unsuspected environmental mutagen of high potency.

A direct search for an increased rate of occurrence of malformations and diseases of genetic origin would necessarily involve a delay of at least 9 months, for any mutation that occurs in the parent will be seen only after the child is born. In the future, intrauterine tests may become feasible; at present the techniques are not adapted to the wide-scale application that would be necessary is a general rise in mutation rate were to be detected. It might be possible to select certain traits that would be the most efficient indicators of an increased mutation rate. Such indicator traits would have to be:

a. Dominant, so the trait shows up in the next generation after the mutation occurs;

b. Present at the time of birth, or shortly after, so that there is no long delay in the discovery;

c. Conspicuous, so that they would be unambiguously and easily detected by those attending the birth;

d. Of a unique appearance not mimicked by other traits not of mutational origin;

e. Of such a nature that it is easy to distinguish new mutants from those cases where the parent had the trait and transmitted it to the child. This latter point could be ensured by having the trait of such a nature as to lead to sterility so that every case is a new mutation.

The number of traits that meet these exacting criteria are very limited; we know of none that does absolutely. But there are probably several that come somewhere near. We are not qualified to suggest a specific set, but we think the possibility ought to be investigated further. As an alternative to choosing traits that are so conspicuous and characteristic that they would always be recognized, one might have those attending the birth simply report all instances where the child is abnormal and then have a staff of specialists in congenital anomalies visit each case. The proportion of births with obvious anomalies is in the vicinity of 1 or 2 percent, so by examination of a tiny fraction of all children born the specialist would have an excellent chance of selecting among these those with defects likely to be mutational in origin.

The success of such a system would not only depend on getting good observations at the source, but also on a system of prompt reporting and data analysis so that any trend could be detected promptly. If an increase is detected one could hope to identify the cause by such things as the geographical pattern.

The monitoring of gross abnormalities may be too crude to produce meaningful results. It may be advisable to use refined chemical procedures that can detect changes in the proteins that are the immediate gene products. At present such tests are very expensive, but with increased automation, these may be shortly feasible. The rough and ready and the refined methods are not mutually exclusive; both have their advantages. We think it is likely that as our chemical environment becomes increasingly more complicated that more and more elaborate systems of monitoring will be necessary.

The cost of genetic monitoring such as we have been discussing would be very great. It could probably be justified only if it were a part of a system of monitoring for other environmental factors. A natural one to couple with a mutation-detecting system would be a search for new teratogens in the environment. Our memory of the thalidomide disaster is a reminder of the need to have a system that will reveal as promptly as possible any agent that is causing physical abnormalities and disease, whether this be by increased mutation or any other cause.

Another possibility for monitoring is to study the human population, as before, but instead of looking at the next generation look at this generation for changes that might foreshadow such changes in the future. If mutations are induced in the germ cells, they are also induced, in all probability, in other body cells. Therefore, a sensitive system of monitoring mutation rates in the blood cells could give a much quicker indication of an environmental change. Such tests could be both chemical tests for altered gene functions and cytological observations for chromosome aberrations.

Conclusions

A number of procedures are presently available in mammals, the majority of recent origin, that can be used to determine the mutagenic activity of chemicals. Our ability to characterize mutagenic agents no longer depends exclusively on nonmammalian systems, such as *Drosophila*, bacteriophage, micro-organisms, and cell culture, although these procedures should be considered as ancillary to the available mammalian tests. The mammalian tests which should be considered as the basis for evaluating potentially mutagenic agents are the host-mediated assay, cytogenetic studies, and the dominant-lethal test. These procedures are as relevant to man as any other animal procedure presently used in the field of toxicology. They are also practical. The dominant lethal test can be concluded in less than 3 months, whereas cytogenetic studies and the host-mediated assay can be carried out in a few weeks. The cost of these tests is considerably less than that of many of the procedures currently used in chronic toxicity testing. It is anticipated that a testing protocol, relying on both the outlined mammalian tests, and the ancillary procedures, should detect the majority of mutagenic chemicals.

Since the mammalian procedures presently recommended are of comparatively recent origin, continued improvements in these techniques can be anticipated. Most important is the need for inexpensive and sensitive tests that can detect point mutations in mammals. Particularly promising in this connection is the development of systems in mice that combine genetically marked chromosomes with crossover-suppressing inversions, which can be used to detect recessive lethal mutations.

A Recommended Program for Mutagenesis Testing

There are several bases for choosing which pesticides are likely to be mutagenic and which need most to be tested. Clearly, it is most important to test those that are used on a wide scale and to which large numbers of humans are exposed. In this context, pesticides that are used in the home are more important than those that are used in areas away from humans and human crops.

It is possible to make some predictions as to which pesticides are most likely to be mutagenic. Substances which are known to be teratogenic, or carcinogenic, or which interfere with reproductive functions are often mutagenic. Chemical structure sometimes can be a useful guide to predicting possible mutagenicity. For example, many alkylating chemosterilants could have been predicted to be mutagenic in advance of actual tests.

If priorities are needed, we would put at the top of the list those pesticides that are used in the largest amounts, with the greatest emphasis on those used domestically and on food crops. Particular attention must be directed to domestic exposure by inhalation of pesticide aerosols and vaporizing pesticide strips. The possibility exists that this may represent a major source of, hitherto unsuspected, human exposure. It should also be stressed that labile pesticides, such as

Captan with a half life of 10 seconds in serum, pose as potentially serious, although perhaps less obvious, mutagenic hazards as do persistent pesticides.

There are only about 400 substances commonly incorporated in current pesticide formulations (*1*). It is feasible to test all of these, using mammalian and ancillary procedures recommended in this report within a reasonable period of time, say, a year.

Although we cannot foresee all contingencies, we recommend the following as a general feasible protocol:

a. Test all compounds now use in the following:

1. Three mammalian systems, the dominant lethal, host-mediated, and *in vivo* cytogenetics, by appropirate routes of administration, reflecting human exposure, and also parenterally, and at high-dose levels, such as maximal tolerated doses.

2. In ancillary microbial systems, preferably those detecting both single nucleotide changes and effects involving more than one gene.

The precision of testing, both in mammalian and ancillary systems, would be such that a doubling of the control level of mutation would be statistically significant at the 5 percent level. A pesticide is regarded as negative if none of the tests is significantly different from its control. If one or more of the three mammalian tests shows a significant effect, the test is regarded as positive. If only the microbial test is positive, more detailed mammalian tests are indicated.

b. If the compound is inactive in all systems, then it is tentatively assumed to be safe. If the compound is widely used or if for any reason there is the possibility of extensive human exposure, it is advisable that more extensive tests be made. Those compounds which have the greatest chance of having an effect on man should be additionally tested, to take into account problems of possible interactions and duration and rate of exposure.

c. If the compound is mutagenic, a reasoned decision must be made as to whether the benefit is great enough to warrant further detailed evaluation, with appropriate interim restrictions on use, or whether its use must be disallowed forthwith.

d. The testing procedures recommended above must be constantly updated and improved to reflect new techniques and new data. We therefore recommend further that a group of disinterested, scientifically competent persons be assigned the problem of continuously reviewing the whole question of pesticide mutagenesis and test systems to be employed.

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BASIC CONSIDERATIONS

Structure-activity relations

Most pesticides have not been designed with the aim of attacking the hereditary material of cells, but their activity has been discovered by chance or they have been designed as analogs of known metabolic inhibitors or activators (*1, 6, 9*). If their assumed metabolic effect is actually responsible for their pesticide action, it should be possible to replace mutagenic by nonmutagenic compounds. The known or presumed major mode of action of pesticides as follows:

1. *Plants* (*herbicides, fungicides*).—

a. Inhibition of photosynthesis (triazines; substituted ureas; carbamates; bipyridylium quarternary salts).

b. Inhibition of oxidative phosphorylation (dinitrophenol analogs, such as toluidines; carbamates).

c. Hormone (auxin) analogs (*2,4-D; 2,4,5-T;* benzoic acid analogs; perhaps maleic hydrazide).

d. Inhibitor of panthothenate synthesis (chlorinated aliphatic hydrocarbons).

e. Inhibitor of porphyrin, hence chlorophyll synthesis (amitrol), which also inhibits purine synthesis.

f. Unknown mechanisms (metals, sulfur).

2. *Animals* (*insecticides, nematocides*).—Most of these are nerve poisons.

a. Inhibitors of acetylcholinesterase (organophosphates, carbamates).

b. Inhibitors of neuromuscular junction (nicotinoids).

c. Neurotoxicants with only partially known causes (chlorinated or brominated hydrocarbons, pyrethroids).

An exception to the general rule are chemosterilants that are designed to produce dominant lethal mutations in insects giving rise to nonviable offspring (*7, 8*). In the United States of America, chemosterilants are not registered for use as pesticides. However, the Entomology Division of the USDA is currently conducting experimental field studies, in some of which the possibility of human exposure cannot be excluded. More alarming is evidence of active commercial interests in chemosterilants in Japan where extensive field tests are now in progress (*4*). It should be emphasized that chemosterilants must never be employed outside the laboratory except under rigorously supervised conditions.

Most reactions which alter the hereditary information in a cell seem to be caused by a chemical or enzymic attack on DNA itself. The major exception to this rule is the effect of colchicine which inhibits spindle formation and causes the production of polyploids. Agents that alter DNA itself produce either mutagenic or inactivating DNA alterations. Mutagenic DNA alterations are minor alterations of the DNA bases which do not prevent DNA duplication but which cause a change in the base sequence of DNA. Such DNA alterations are induced by base analogs (2-aminopurine, 5-bromouracil) which are incorporated into DNA or by the chemical alterations of DNA bases such as the deamination of adenine or cystosine by nitrous acid, the hydroxylamination of cytosine by hydroxylamine, the alkylation of guanine by alkylating agents, or the intercalation of acridine dyes between DNA bases. Mutagenic DNA alterations give rise to point mutations.

In contrast, inactivating DNA alterations have more drastic effects on DNA, since they inhibit the duplication of DNA across the altered side. Such alterations arise when a DNA base is removed or the DNA backbone is broken as a consequence of treatment by alkylating agents, radical-producing agents, or base analogs which inhibit the duplication of DNA. Many inactivating DNA alterations can be repaired by special cellular enzymes. Different organisms differ in the extent and the specificity of repair mechanisms. DNA alterations that have not been repaired lead to chromosomal breaks which are usually lethal to the cell. If more than one chromosome break occurs within one cell, large heritable chromosome aberrations can be produced (deletions, translocations, inversions, etc.). Most, if not all, agents which induce inactivating DNA alterations or chromosome breaks *in vivo* have also been found to induce mutations, cancer, and teratogenic effects, when examined in the proper test system (*2, 3, 5*).

Most compounds affecting DNA produce both mutagenic and inactivating DNA alterations but the relative frequency of these two effects differs up to one million-fold for different compounds (*2, 3*). A mutagenic test system which is very sensitive for one compound, *e.g.*, transition type point mutations, may therefore reveal no mutations with another compound that produces only other types of mutations, *e.g.*, large chromosome alterations. There is also no correlation between toxic and mutagenic effects because some highly mutagenic compounds, *e.g.*, certain base analogs, are barely toxic, whereas some highly toxic compounds, such as cyanide, are hardly mutagenic.

On the basis of theoretical extrapolation from available data, pesticides may be classified in three major groups by chemical structure:

A. Compounds known to alter DNA directly in some biological system or compounds having chemical structures that are known to alter DNA: alkylating agents, radical-producing agents, inhibitors of DNA synthesis. Irrespective of any further tests, on these compounds, extreme care with respect to human exposure is recommended.

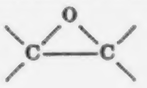
B. Compounds which by their structure may possibly affect DNA either directly or after enzymic activation into reactive compounds. Among these are mercurials, some of which are known mutagens, and carbamates, some of which may be converted by plant and animal systems into N-hydroxycarbamates that produce chromosome breaks. Due to problems of uptake, enzymic activation or inactivation, and accumulation, it is not possible to make any safe prediction of the mutagenicity of these compounds in mammals. But thorough testing is necessary.

C. Not suspected to produce genetic alterations because their chemical reactivity with DNA or their mutagenicity have not been tested and their structure does not suggest such activity. Among the unsuspected chemical structures are cyclopropane rings, as found in pyrethrins, triazines, *2,4-D* and other auxin analogs, and those chlorinated hydrocarbons which do not belong to group A or B (*e.g.*, DDT). Nevertheless, our general ignorance concerning metabolic conversions makes it desirable that all these compounds be also tested for mutagenicity. Some triazines *e.g.*, Aziridines also have alkylating groups and for that reason belong in group A.

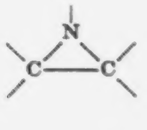
Chemical structures known or suspected to affect DNA and pesticides having these structures are indicated as follows:

A. *Compounds having chemical structures that are known to affect DNA. Any compound having such a structure should be proven to be harmless before humans are exposed to it.*

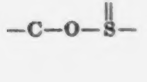
a. *Alkylating agents*, induce both point mutations (transitions) and large chromosome alterations.



1. Epoxides (Ethylene oxide, Endrin, Dieldrin).



2. Ethyleneimines (Aziridines, such as Apholate, TEPA, thio-TEPA, METEPA).

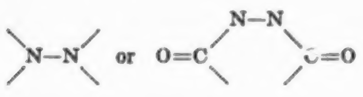


$-\mathrm{C}-\mathrm{O}-\overset{\mathrm{O}}{\overset{\|}{\mathrm{S}}}-$

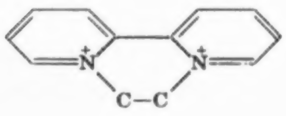
3. Sulphates (Aramite).

4. Certain Bromides and Chlorides (Bromomethane, Bromopropane, Dichloroethane, Ethylene dibromide, Propargylbromide).

b). *Radical producing agents*, induce large chromosome alterations but not point mutations.



1. Hydrazines or hydrazides (Maleic hydrazide, known to break chromosomes).



2. Bipyridylium quarternary salt (Diquat, Paraquat). Known to produce radicals and to kill plants in the presence of oxygen (presumably via peroxide radicals affecting DNA).

c). *Base analogs*

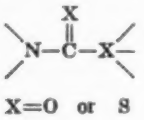
5-Fluorouracil, fluoroorotic acid; both DNA synthesis.

B. *Compounds suspected to affect DNA or to be converted enzymatically into effective compounds. These pesticides must be tested for their mutagenicity in higher organisms.*

a). *Unsaturated rings with -OH or SH groups.* Some phenols and cresols are known to produce chromosome breaks perhaps due to radical formation in presence of oxidizing groups.

(Ioxynil, Niacide, Orthophenylphenol, PCP, etc.)

b) Carbamates and Thiocarbamates



(Barban; Bux Ten; Carbaryl; Carbofuran; Carzol; CDAA; Dimetilan; Metham; Mobam; Propachlor; 2,3,6 TBA; Temik; Thiram; Vapam; Ziram; and many others).

Ethylcarbamate and several other carbamates are well-known to produce chromosome breaks, cancer, and teratogenic effects. They do not affect DNA directly but their enzymic products, such as N-hydroxycarbamates and other intermediates which in turn produce radicals, cause inactivating DNA alterations; thus, chromosome breaks and large chromosome alterations, but not point mutations, can be induced. Whether or not a particular carbamate is mutagenic depends therefore on the presence of the N-hydroxylation (or other) enzymes in the cells.

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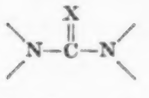
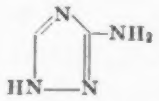
c. *Ureas*



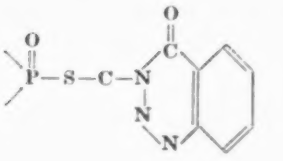
X=O or S

(Chloroxuron, DCU, Diuron, Fenuron, Linuoron, Norea, etc.) These compounds also alterations if they are activated N-hydroxylation).

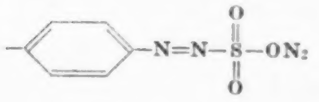
d. *Compounds having 2 or 3 nitrogens connected.* If they should be known to produce alkylating agents (certain nitroso compounds) or radicals, they should be placed in Group A.



Triazoles (Amitrole)



Benzotriazines (Azinphosethyl, Azinphosmethyl)



Diazo compounds (Dexon, also alkylating?)

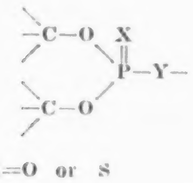
e. *Mercurials*

—Hg—

(Elcide, Emmi, Ethylmercury chloride, Panogen, Ceresan, Semesan Bel, and others)

Some mercurials are known mutagens, presumably owing to their content of Hg.

f. *Organophosphates*



X=O or S

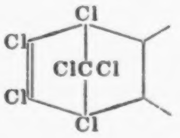
Y=O—, S—, or N<

(Parathion, Methylparathion, Malathion, Demeton, Disulfotone, Dimethoate, HMPA Phorate, Phosphamidone, Cyolane, Dimefax, Monitor, Ruelene and many others). These compounds are phosphate triesters which are labile to hydrolysis. Although not tested, some of them may react with DNA by transalkylation.

g) *Other reactive compounds*

(Acrolein, Allyl alcohol, Acrylonitrile [known to react with 4-uridine, inosine, t-RNA])

h) *Chlorinated cyclodienes* having an unsaturated group in a ring attached to the chlorinated one.



(Aldrin, Isodrin, Heptachlor)

These compounds are known to be converted into epoxides that are found in body fat.

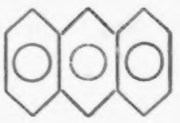
i) *Base analogs*

(Benlate, Isocil, Lenocil, Lavozil, Bromacil, Terbacil)

Some of these compounds may possibly inhibit DNA sysnthesis.

j) *Arsenates, Cacodylic Acids,* inhibit phosphorylation

k) *Potential Intercalating Compounds*



(Anthraquinone, Morestan, Phenothiazine)

Some of these compounds may act similar to acridines and intercalate between DNA bases.

l) *Certain antibiotics*

(Griseofulvin)

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Usage patterns

The expected increased need for pesticides in the next 5–10 years will result in the greater use of currently registered chemicals. New products are also being rapidly developed and will no doubt replace a number of widely used pesticides. The enclosed summary lists the major categories of pesticides that predominate the market.

Herbicides.—Preemergence herbicides, such as atrazine (triazine derivative), trifluaralin (a dinitrotoluidine), amiben (benzoic acid derivative), lead the use of herbicides and are expected to continue to grow over the next several years.

Postemergence herbicides, such as phenoxyacetic acid derivatives (i.e., 2,4-D, 2,4,5-T), contribute greatly to the use of pesticides and represent a major use category.

Insecticides.—For years DDT, along with other chlorinated hydrocarbons, dominated the insecticide market. However, due to their persistence and potential ecological and human hazards, they will probably be phased out of agricultural use in the U.S. in the early future. Malathion, a phosphate insecticide, will likely increase in usage over the next several years and represents a major class of insecticides in use. Carbaryl, a carbamate insecticide, is expected to continue to grow in use and is now a major insecticide. Similarly, the systemic insecticides, such as carbofuran, dimethoate, disulfoton, methyl-demeton, phorate, phosphamidon, are major products in use today.

Based on U.S. sales at manufacturing levels, the following materials can be considered the most widely used in agriculture:

Herbicides:

Atrazine
Trifluralin
Amiben and related products
2,4-D
2,4,5-T
Nitralin
Propachlor, CDAA and related products
Picloram
Paraquat
Dicamba

Insecticides:

Carbaryl
Malathion
Aldrin
Diazinon
Toxaphene
DDT
Methyl parathion
Parathion
Azinphosmethyl
Chlordane
Heptachlor
Disulfoton
Phorate
Kelthane
Bux Ten
Endrin

Fungicides:

Dithiocarbamates
Captan
Pentachlorophenol
Dodine

Fumigants:
Methyl bromide

Of these agriculturally important materials, several should be given extra consideration because of their widespread usage in household formulations. Among these are chlordane, DDT, and its analogs, BHC and other chlorinated hydrocarbons, including the cyclodienes, and certain organophosphates such as Malathion. In addition, a number of other products are used particularly for household purposes, especially in aerosols and vaporizing strips, and should consequently be considered as priority compounds for mutagenic evaluation. Included in this group are the pyrethroids and their synergists, i.e., piperonyl butoxide, Dichlorvos, and such commonly applied insect repellants, such as diethyltoluamide, and ethylhexanediol. Some of these materials are particularly important, because human exposure occurs largely through inhalation via aerosol sprays or vaporization or skin absorption, and to a lesser extent also by ingestion.

LITERATURE SUMMARY

General considerations. Approximately 400 chemicals are now used in the control of weeds, insects, nematodes, rodents and plant diseases(*1*). In the present literature search, more than 500 published papers on the mutagenicity of pesticides have been located. Many of these papers refer to compounds which are not currently in common use in the United States, but which in some cases are used elsewhere. Therefore, they represent a potential hazard to the population of the United States, either as contaminants in imported foodstuffs, or through future registration in the United States. In this preliminary manual literature search, we located 42 papers referring to mutagenic testing of some compounds in a recent listing (*1*). In total, 31 of the 32 compounds tested showed mutagenic activity in at least one system. It should be stressed, however, that this represents a group of compounds preselected as likely to be mutagenic. We have no doubt that a more extensive search among the literature already in Environmental Mutagen Information Center (EMIC) files would reveal that many more of the commonly used pesticides possess mutagenic activity.

It is apparent from the literature that there has been no large scale testing of pesticides for mutagenic activity. Existing reports are therefore sporadic. Most of the tests for mutagenic activity of pesticides have been done on plants.

A detailed summary table of literature reviewed is given in the appendix. The following examples are presented for illustration only:

a. *Fumigants.*—These are generally used in restricted areas. The compounds will only reach the human population if they are persistent. As fumigants are often highly reactive, it is likely that their breakdown products may reach the general population. For example, ethyleneoxide is a highly reactive alkylating agent which has been shown to be mutagenic in many systems. If chlorine ions are present in fumigated material, ethylene chlorohydrin will be formed. This compound, stable enough to persist in marketed material, induces point mutations in *Neurospora crassa.*

Several other fumigants which have shown mutagenic action include formaldehyde, active in *Neurospora* and *Drosophila*, and ethylene dibromide, active in *Neurospora.*

b. *Mercury pesticides.*—Twelve of the 317 pesticides in a recent listing (*1*) contain mercury. Eight hundred thousand pounds of mercury, representing 15 percent of the total amount of mercury marketed *per annum* in the U.S., are used in the production of pesticides. Most of the remaining 85 percent is used in mildew-resistant paints. In nature, most mercurial compounds are finally converted into methylmercury. This accumulates in fish and shellfish. Human consumption of such seafood may lead to accumulation of methyl-mercury to even lethal levels. Methyl-mercury causes nondisjunction in *Drosophila;* high levels of chromosome aberrations have been found among heavy fish eaters in Sweden (*2*). In Japan, deaths and teratological effects have been directly attributed to the intake of mercury containing seafoods (*3*). It is known that mercurial residues can persist up to 100 years in polluted lakes. The use of many mercury pesticides is now prohibited in Sweden.

c. *Organophosphate insecticides.*—Many are triesters of phosphoric acid, and as such might be alkylating. The simplest triester is trimethylphosphate, while not, however, a pesticide, can induce point mutations in *Nuerospora crassa* and is highly active in the dominant lethal mouse test (*4*).

d. *Miscellaneous.*—Captan induces chromosome rearrangements in rats and point mutations in *Nuerospora crassa.* Maleic hydrazide breaks chromosomes in many plant systems, although inactive in the dominant lethal test in the mouse. Lindane is also similarly active in many plants systems.

Conclusion.—Although only limited information on the mutagenic action of commonly used pesticides exists at present in the literature, it is likely that automated procedures will be required to keep up with an expanding literature.

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Appendix

BIBLIOGRAPHY

The literature listings compiled in this search were obtained manually or through MEDLARS. This literature search should still be considered incomplete. In a few cases, key words have been added after the literature references. The literature references have been subdivided into the following categories:

Induction of mutation, chromosomal effects, antimitotic effects, and other effects related to mutagencity.

Teratogenic effects.

Biochemistry.

DDT and related compounds, papers not included in the earlier categories.

Distribution.

Reviews and symposia.

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LITERATURE REVIEW

These data have been collated from files of the Environmental Mutagen Information Center and are presented for illustrative purposes only. They are not presented as comprehensive listings.

Pesticide [1]	Organism in which tested	Assay system	Dose		Biological effect	EMIC registry No.
			Range	Minimum effective dose		
Acrylonitrile	Yeast	t-RNA			Cynoethylation	569
Do		do			Pseudo-uridine modification	568
Atrazine	Barley	Anther	1,000 p.p.m.—Soaked		Slight effect on meiosis (C_1)	70
					Slight effect on meiosis (C_2)	70
Captan	Human embryo	L-132 cells	10 mcg/ml		Inhibition of DNA synthesis	426
Do	Mice	Sperm	9 mg./kg 500 mg./kg.		Negative induction of dominant lethals.	23
Do	Rat kangaroo	Somatic and germ cells.	1.25 to 5.0 mcg./ml		Chromosome aberrations	426
Carbaryl	Barley	Anther	1,000 p.p.m.—Soaked		No effect on meiosis (C_1)	70
			500 p.p.m.—Sprayed		Abnormal meiosis (C_2)	70
Do	Plant	Root tips	0.5 and 0.25		Abnormal mitosis	1
			Saturated		Chromosome Aberrations.	
Chloroform	*Allium cepa*	do	Saturated to 0.005%	0.025%	C-mitosis chromosome aberrations.	553
Do	do	do			C-mitosis	535
Chlorphrophan		Plant cells	2.5, 5, 10, 20, 40, 80 p.p.m.		C-mitotic effect Nuclear disintegration.	95
2,4-D	Narcissus	Root tips	0.01, 0.05, 0.1%		C-mitosis Chromosome aberrations.	391
2,4-D	Cotton (Acla 44)	Cotyledons	10^{-3} to 10^{-4} M		Effects nucleic acid synthesis	277
2,4-D	*Allium cepa*	Root tips	0.01, 0.05, 0.1%		C-mitosis Chromosome aberrations.	391
2,4-D	*Vicia faba*	do	0.001% to 1.0%	0.001%	Abnormal mitosis	60
2,4-D	*Allium cepa*	do	25 to 500 ppm	25 ppm	Chromosome aberrations	291

Pesticide [1]	Organism in which tested	Assay system	Dose		Biological effect	EMIC registry No.
			Range	Minimum effective dose		
2,4-D	Tradescantia		0.001% to 1.0%	0.001%	Abnormal mitosis	60
DCNA	Barley	Anther	1,000 p.p.m.—Soaked		Slight effect on meiosis (C_1)	70
			500 p.p.m.— Sprayed		High abnormal meiosis (C_2)	70
DDT	Mice	Sperm	105 mg./kg		Negative induction of dominant lethals.	23
DDT	*Allium cepa*	Root tips	Saturated solution		C-mitosis and chromosome breaks.	408
DDT	*Trigonella foreum graecum.*	do	Saturated solutions		do	408
Dichlorvos	Onion	do	0.5 to 6.0 sq. cm		Chromosome breaks	396
Dicomba	Barley	Anther	1,000 p.p.m.—Soaked		Abnormal meiosis (C_1)	70
			500 p.p.m.—Sprayed		Abnormal meiosis (C_2)	70
Dieldrin	*Crepis capillaris*	Sprouts	10% solution		C-mitosis effect, no chromosome breaks observed.	40
Endothall		Plant cells			Chromosome aberrations	570
Endrin	Barley	Anther	1,000 p.p.m.—Soaked		No effect on meiosis (C_1)	70
			500 p.p.m.—Sprayed		No effect on meiosis (C_2)	70
Ethylene oxide	Fungi		0.025 M		Point mutations and reverse mutations.	258
Do	*Neurospora crassa*	Conidia	0.14 M		Point mutations and reverse mutations.	34
Do	Maize	Plant cells	1 part E.O. to 20 parts air.		Chromosome breaks	25
Ethylmercury chloride.	Triticum	Root tips	0.5 to 1%		Mitotic aberrations	357
Do	*Secale cereale*	do	do		do	357

Ferbam	*Aspergillus niger*	Spores	1,000 p.p.m.		Morphological mutants and reverse mutations.	49
Do.	*Allium cepa*	Root tips	240 p.p.m.		Chromosome aberratlns	49
Formaldehyde	*Drosophila melanogaster.*	Sperm	0.033–0.05 M		Induced very low incidence of recessive lethals but enhanced the effect of X-rays.	404
HCN	Mammalian embryo.	Heart, spleen and liver cells.	0.20×10^{-2} to 0.88×10^{-6}.		Some nuclear abnormalities induced.	460
Do.	*Vicia faba*	Root tips	4×10^{-4} M.		Chromosome breaks	510
Isocil	Barley	Anther	1,000 p.p.m.—Soaked		Abnormal meiosis (C_1)	70
			500 p.p.m.—Sprayed		Abnormal meiosis (C_2)	70
KOCN	*Vicia faba*	Root tips	400 µM/L. 1,000 4M/L.		Chromosome breaks.	
Lindane	Onion	do	1/20,000 to 1/80,000		do	396
Do.	*Allium cepa*	do	0.00125%		Induced aneuploidy and chromosome fragmentation.	478
Do.	do	do	2 to 0.0006%	0.00125%	Induced C-mitosis	571
Do.	*Zea mays*	Root tips, stems Coleoptile tissue.	Solid particles		Chromosome aberrations	502
Do.	*Triticum vulgare*	do	do		do	502
Do.	*T. Monococcum*	do	do		do	502
Do.	*T. compactum*	do	do		do	502
Do.	*Secale cereale*	do	do		do	502
Do.	*Setaria Hatica*	do	do		do	502
Do.	*Helianthus annus*	do	do		do	502
Do.	*Crepis capillaris*	do	do		do	502
Do.	*Vicia faba*	do	do		do	502
Do.	*V. sativa*	do	do		do	502
Do.	*Brassica nigra*	do	do		do	502

Pesticide [1]	Organism in which tested	Assay system	Dose		Biological effect	EMIC registry No.
			Range	Minimum effective dose		
Linuron	Rhizobium	Cells	0–500 μg./ml.	200 μg./ml.	Forward mutation induction	32
Do.	Barley	Anther	1,000 p.p.m.—Soaked		No effect on meiosis (C$_1$)	70
			500 p.p.m.—Sprayed		No effect on meiosis (C$_2$)	70
MH		Plant cells	10^{-4} M		Chromosome breaks	33
MH	*Vicia faba*	Root tips	do		Chromosome breaks, suppresses mitosis.	517
MH	do	do	do		Chromosome breaks	19
MH	do	do	0.0005 to 0. 0001 M		Chromosome aberrations	19
MH	do	do			Chromatid breaks	24
MH	*Drosophila melanogaster*.	Sperm	0.4%		No recessive lethal induction	378
MH	Guinea pig	Tissue cultured ear cells.	0.01 M		No morphological effect	6
MH	Tomato	Root tips	10^{-2} M, 10^{-3} M, 10^{-4} M.	10^{-4} M	Chromosome aberrations	329
MH	Mice	Sperm	500 mg./kg		Negative induction of dominant lethals.	23
Monuron	Barley	Anther	1,000 p.p.m.—Soaked		Abnormal meiosis (C$_1$)	70
			500 p.p.m.—Sprayed		Abnormal meiosis (C$_2$)	70

Naptalam	do	do	1,000 p.p.m.—Soaked		Abnormal meiosis (C_1)	70
			500 p.p.m.—Sprayed		Abnormal meiosis (C_2)	70
Paradichlorobenzene.	*Vicia faba*	Root tips	Saturated solution		Abnormal mitosis	64
					Chromosome breaks	64
					Chromosome fragmentation	64
Parathion	*Allium cepa*	do	0.01, 0.005, 0.0075%		Induced C-mitosis	476
PCP		Plant cells	Saturated solution		Meiotic effect	2
Phosphamidon	Barley	Anther	1,000 p.p.m.—Soaked		Slight effect on meiosis (C_1)	70
			500 p.p.m.—Sprayed		Slight effect on meiosis (C_2)	70
Propham	*Avena sativa*	Root and stem tips.	0.1 to 5.0 p.p.m		Mitotic aberrations	314
Do		Plant cells	2.5, 5, 10, 20, 40, 80 p.p.m.		C-mitotic effect	95
Do	Plant cells	do	do		Nuclear disentigration.	
Do	Avena and allium	do			Anaphase bridges, blocked, nuclear fragmentation.	312
Simazine	Barley	Anther	1,000 p.p.m.—Soaked		Slight effect on meiosis (C_1)	70
			500 p.p.m.—Soaked		No effect on meiosis (C_2)	70
2,4,5-T	*Allium cepa*	Root tips	25 to 500 p.p.m	25 p.p.m	Chromosome aberrations	294
2,4,5-T	Apricot	Fruit cells	100 mg./L		Slight antimitotic effect	445

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[1] Name according to Neumeyer et al., "Chemical Week," Apr. 12 and 26, 1969.

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EMIC registry No.	Reference
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378	Nasrat, G. E.: Maleic hydrazide, a chemical mutagen in *Drosophila relanogaster*. Nature 207: 439. 1965.
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396	Sax, K., and H. Sax.: Possible Mutagenic Hazards of Some Food Additives, Beverages, and Insecticides. Japan J. Gen. 43(2); 89–94. 1968.
404	Sobels, F. H.: The effect of formaldehyde on the mutagenic action of X-rays in *Drosophila*. Experientia 12(8): 318. 1956.
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460	Danes, B., and P. Leinfelder: Cytological and Respiratory Effects of Cyanide on Tissue Cultures. J. Cell. Comp. Phys. 37: 427–446. 1951.
476	Gimenez-Martin, G., and J. Lopez-Saez: Accion a-Mitotica del Parathion. ΦΥΤΟΝ 18: 23–26. 1962.
478	Gimenez-Martin, G., and J. Lopez-Saez: Acction del gamma-hexaclorociclo-hexano sobre la division celular. ΦΥΤΟΝ 16: 45–55. 1961.
502	Kostoff, D.: Induction of cytogenetic changes and atypical growth by hexaclorocyclohexane. Science 109: 467–468. 1949.
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568	Yoshida, M., and T. Ukita: Selective Modifications of Inosine and Ψ-Uridine with Acrylonitrile out of the Other Ribonucleosides. J. Biochem. 57(6): 818–821. 1965.
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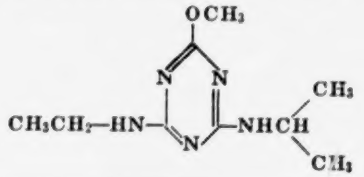
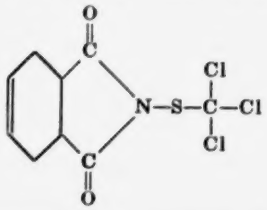
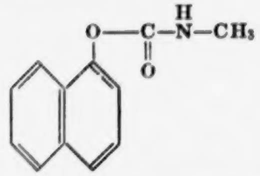
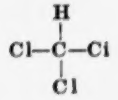
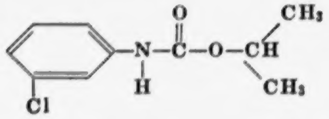
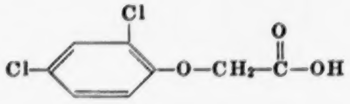
Addition Data on Cited Compounds*

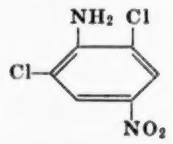
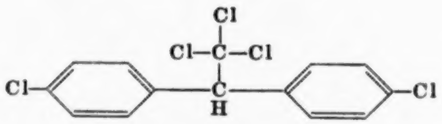
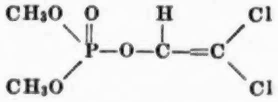
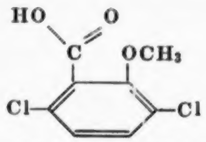
Products, producers	U.S. patents	Chemical name and formula	Physical properties	Product form	Oral toxicity LD_{50}	Major end-uses
	Fu	Acrylonitrile				
Acrylonitrile Acritet* Stauffer Carbide Monsanto Cyanamid		$CH_2{=}CHCN$	Colorless liquid b.p. 77.3–77.5 C	V	93	Fumigant for stored grain, tobacco, nuts, and dates to control insects
	H	2-(Ethylamino)-4-(isopropylamino)-6-methoxy-s-triazine				
Atratrone Gesatamin G–32293 Geigy			Colorless, crystalline solid m.p. 94–96 C		1,465	Experimental herbicide; absorbed by both leaves and roots, unlike simazine, which is taken up only by roots
	F	N-Trichloromethylthio-4-cyclohexene 1,2-dicarboximide				
Captan* Orthocide* Stauffer Chevron	2,553,770 2,553,771		Colorless to buff powder m.p. 158–164 C	WP D	10,000	As a protectant-eradicant fungicide for fruits, vegetables and flowers in control of scabs, blotches, rots, mildew, etc.

APPLICATION: F, fungicide; **Fu,** fumigant; **H,** herbicide; **I,** insecticide; **L,** larvacide; **M,** miticide; **Mo,** molluside; **N,** nematocide; **R,** repellant; **Ro,** rodenticide; **S,** synergist; **PGR,** plant growth regulator. **PRODUCT FORM: A,** aerosol; **B,** bait; **C,** concentrate; **D,** dust; **EC,** emulsifiable concentrate; **G,** granules; **OS,** oil solution; **P,** powder; **S,** spray **ULV,** ultralow volume; **V,** vapor; **WML,** water miscible liquid; **WSC,** water-soluble concentrate; **WP,** wettable powder.

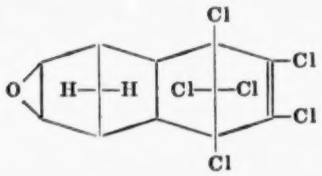
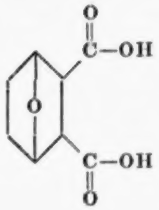
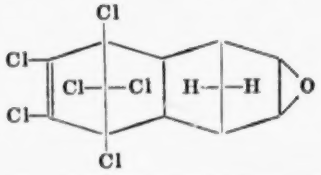
*Table taken from Neumeyer, et al. (op. cit.)

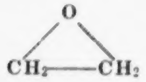
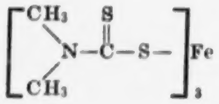
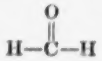
Addition Data on Cited Compounds*

Products, producers	U.S. patents	Chemical name and formula	Physical properties	Product form	Oral toxicity LD_{50}	Major end-uses
I Carbaryl Sevin* Carbide	2,903,478	1-Naphthyl N-methylcarbamate 	Colorless, crystalline solid m.p. 142 C d_{20}^{20} 1.232	WP D G S	540	Control of insets on fruits, vegetables, forage, cotton and other economic crops, as well as poultry and pets
Fu,I Chloroform		Chloroform  $\mathrm{Cl{-}\overset{H}{\underset{Cl}{C}}{-}Cl}$	Colorless, liquid b.p. 60–61 C			Insecticide; grain fumigant mixture contains 73.2% $CHCl_3$, 26.8% CS_2; screw worm control on animals
H Chlorpropham Chloro IPC CIPC PPG	2,695,225	Isopropyl *m*-chlorocarbanilate 	b.p. 247 C (decomp.) m.p. 38–39 C	EC G	5,000–7,500	Highly selective preemergence and early post-emergence herbicide; effective control of many annual grassy and broadleaved weeds
H 2,4-D Verton* D DMA-4* WEEDAR* Monsanto Hercules Chipman Thompson-Hayward Diamond Dow		2,4-Dichlorophenoxyacetic acid: also used as amine salts and esters 	Colorless powder m.p. 138 C	EC G WML	500	Plant growth regulator; post-emergence weed control in cereal grains, corn, pastures and lawns; aquatic weeds

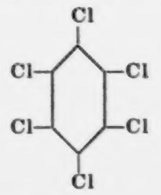
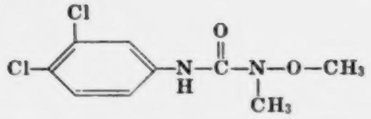
DCNA Dichloran Botran* Ditranil Tuco	**F**		2,6-Dichloro-4-nitroaniline 	Yellow m.p. 192–194 C	WP D	10,000	Selective fungicide used as soil treatment or foilage spray or dust; particulary effective against [illegible] *trytis*, *Schlerotinia*, *Mon*[illegible] *lerotium* and *Rhizopus*
DDT Dichloro-diphenyl-trichloroethane Genitox* Anofex* Chlorophenothane Lebanon Allied Diamond Montrose Olin Geigy	**I**		1,1,1-Trichloro-2,2-bis *p*-chlorophenyl) ethane 	Colorless or cream m.p. 108.5–109 C	EC WP D G	113	Broad-spectrum insecticide for fruits, vegetables, cotton, household, livestock, timber and industrial use
Dichlorvos Vapona* DDVP Herkol* Dedevap* Oko* Mafu* Shell	**I**	2, 956, 073	2,2-Dichlorovinyl dimethyl phosphate 	Colorless to amber b.p. 77 C/1 mm.	EC G S B	56–80	Control certain insects that are economically important in public health (man and livestock) and insects that attack stored products; effective against household pests
Dicamba Banvel* D Velsicol	**H**	3, 013, 054	3,6-Dichloro-o-anisic acid 	Light-tan, granular solid m.p. 114–116 C	WSC	3, 500	For control of annual broad-leaved weeds in fall and spring, seeded small grains, established perennial grasses, golf course fairways and greens; pre- and post-emergence weed control in field corn, unwanted brush

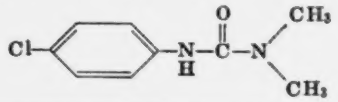
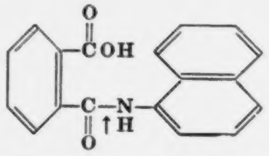
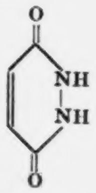
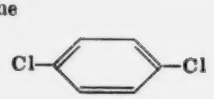
Addition Data on Cited Compounds*

Products, producers	U.S. patents	Chemical name and formula	Physical properties	Product form	Oral toxicity LD_{50}	Major end-uses
	I	Not less than 85% of 1, 2, 3, 4, 10, 10-hexachloro-6, 7-epoxy-1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-1, 4-endo-exo-5, 8-dimethanonaphthalene				
Dieldrin Octalox* Panoram D-31* Shell	2, 676, 131		Buffto light-brown m.p. 175-176 C	EC WP D G	60	To control general soil-inhabitating insects and certain insects attacking principal field, vegetable and fruit crops; mothproofing; public health pests; disease vectors; broad-spectrum insecticide
	H	7-Oxabicyclo (2.2.1) heptane-2,3-dicarboxylic acid				
Endothall Aquathol Hydrothal Accelerate Des-I-Cate Herbicide 282* Herbicide 273* Pennsalt	3, 207, 593 3, 321, 294 3, 246, 015		m.p. 116 C	WSC	51	Pre- and post-emergence herbicide and harvest aid; root crop and vegetable protection; aquatic herbicide; alfalfa and clover desiccant
	I,Ro	1, 2, 3, 4, 10, 10-Hexachloro-6, 7-epoxy-1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-1, 4-*endo-endo*-5, 8-dimethanonaphthalene				
Endrin Shell Velsicol	2, 676, 132		Light colored, free-flowing, crystalline solid	EC WP D G	7. 3-43. 4	Control of pests such as cotton insects, cutworms, armyworms, aphids, corn borer, cabbage looper, grasshoppers, plant bugs, lygus bugs, webworms and many other pests; also used as rodenticide

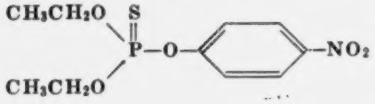
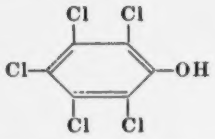
Ethylene oxide Oxirane Dow Jefferson Carbide Wyandotte	**Fu**		Ethylene oxide 	b.p. 10 C m.p. 111 C	V		Fumigant for stored foods
Ethylmercury chloride Ceresan* Granosan* Du Pont	**F**		Ethylmercury chloride $CH_3CH_2—Hg—Cl$	Colorless, crystalline solid m.p. 190-193 C	D		For treatment of cotton, peanuts and pea seeds to control numerous seed-borne diseases and to reduce seed decay and check damping-off; as a short-soak treatment for basal rot of narcissus bulbs
Ferbam Fermate* Vancide-FE95 Du Pont FMC Wood Ridge Vanderbilt Pennsalt	**F**	1,972,961	Ferric dimethyl dithiocarbamate 	Black powder m.p. 180 C (decomp.)	WP	17,000	To control many fungus diseases of fruits and nuts, certain vegetables, tobacco and ornamentals
Formaldehyde Formalin Allied Celanese	**Fu**		Formaldehyde  $H—\overset{\overset{O}{\Vert}}{C}—H$	Colorless gas b.p. −21 C	V	Toxic to plants, animals	Fumigant; soil sterilant (mushrooms) and seed treatment
Hydrogen cyanide Hydrocyanic acid Prussic acid Cyanamid	**Fu**		Hydrocyanic acid $H—C\equiv N$	b.p. 26 C/760 mm.		LD 200 ppm.	Fumigant for raw agricultural commodities, including grain

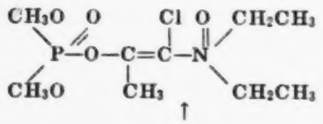
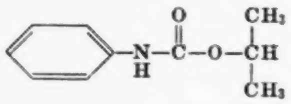
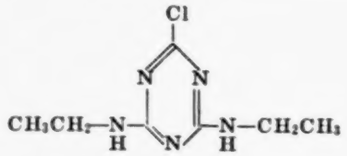
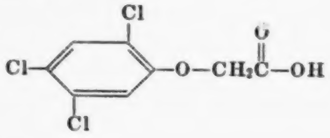
Addition Data on Cited Compounds*

Products, producers	U.S. patents	Chemical name and formula	Physical properties	Product form	Oral toxicity LD_{50}	Major end-uses
KOCN Potassium cyanate Aero* cyanate Weed Killer Cyanamid	**H**	Potassium cyanate K—O—C≡N	Colorless, crystalline needles m.p. 315 C	C	1,000	Foliar spray in controlled emerged annual weeds in onions and certain other crops; control of crabgrass, chickweed and annuals in turf
Lindane gamma BHC Diamond Hooker	**I**	1,2,3,4,5,6-Hexachlorocyclohexane containing at least 99% gamma isomer 	Colorless, odorless crystals m.p. 112.9 C	EC WP D A	90	Broad-spectrum insecticide for apples and other fruits, beans, peas, cole crops, cucurbits, tomatoes, other vegetables; also for dairy, livestock, household and seed-treatment use
Linuron Lorox* Afalon Du Pont	**H**	3-(3,4-Dichlorophenyl)-1-methoxy-1-methylurea 	Colorless, crystalline solid m.p. 93-94 C	WP G	1,500	For selective weed control in corn, soybeans, grain sorghum, cotton, wheat, carrots, parsnips and potatoes; short-term control of annual weeds in noncropland areas such as roadsides and fencerows

Monuron Telvar* Du Pont	**H**	2, 655, 455	3-(*p*-Chlorophenyl)-1,1-dimethylurea 	Colorless, crystalline solid m.p. 174–175 C	WP	3, 600	For selective control of weed seedlings in asparagus, avocado, citrus fruits, cottonseed, grapes, onions, pineapple, spinach
Naptalam Alanap* NPA Dyanap (mixed with DNBP) Uniroyal		2, 556, 664 2, 556, 665 2, 736, 646 2, 736, 647	N-1-Naphthylphthalamic acid 	m.p. 185 C	WP G	1, 770	Selective preemergence herbicide for soybeans, peanuts, sweet potatoes Irish potatoes, cucurbits
MH* Maleic hydrazide MH-30* Slo-Gro* Sucker-Stuff* Retard* Uniroyal Ansul Chem. Form.		2, 614, 916	1,2-Dihydro-3,6-pyridazinedione 	Colorless m.p. 296–298 C	WSC	2, 200	Plant growth inhibitor; blocks cell division for inhibition of grasses, suckers on tobacco; sprout inhibitor for potatoes, onions
Paradichlorobenzene Allied Monsanto Dow PPG	**Fu, I, F**		*p*-Dichlorobenzene 	Colorless crystals m.p. 53 C	C	2, 562	Fumigant against household insects in mothballs; peach tree borers, bark beetles; tobacco blue mold, mildew and other fungi

Addition Data on Cited Compounds*

Products, producers	U.S. patents	Chemical name and formula	Physical properties	Product form	Oral toxicity LD_{50}	Major end-uses
I, M Parathion* Phoskil* Thiophos* Folidol E-605 Niran* Alleron* Alkron Ethyl parathion Corothion* Orthophos Panthion* Parawet* Stathion* Stauffer Cyanamid Monsanto Shell Velsicol Amer. Potash	2,482,063	O,O-Diethyl O-*p*-nitrophenyl phosphorothioate 	Pale-yellow liquid b.p. 157-162 C/O.6 mm. d^{25} 1.265	WP EC D	6-15	Broad-spectrum insecticide effective against aphids, mites, *Lepidoptera*, beetles, leaf-hoppers and thrips on fruits, vegetables and forage crops cotton insects, symphilids, rootworms and other soil insects
F,H,Mo PCP Pentachlorophenol RCI 49-162 Sodium pentachlorophenate Dow Monsanto		Pentachlorophenol 	Buff m.p. 714 C	C WSC	210	Contact herbicide and wood preservative; herbicide and desiccant on sugarcane; molluscicide to control snail carriers of larval human blood flukes causing schistosomiasis

Phosphamidon Dimecron Chevron	**I.M.**	2, 908, 605	2-Chloro-2-diethylcarbamoyl-1-methylvinyl dimethyl phosphate 	Colorless oil b.p. 162 C/1.5 mm.	WP D C	27	Effective against aphids, mites, beetles and plant insests, both systemic and contact
Propham IPC Chem Hoe* PPG	**H**		Isopropyl N-phenylcarbamate 	Colorless crystals m.p. 87-88 C	EC WP D C G	4, 500-9, 000	Selective preplanting and pre-emergence herbicide; effective control of wild oats, many annual grasses and certain broad-leaved weeds
Simazine Princep* Gesapun* Amizine* Primatol S* CDT CET Geigy	**H**	2, 891, 855	2-Chloro-4,6-bis(ethylamino)-s-triazine 	Colorless, crystalline solid m.p. 225-227 C	WP G	5, 000	Controls most annual grasses and broadleaved weeds in corn, sugarcane, fruits, nuts, asparagus and turf
2,4,5-T* Diamond Dow Hercules Millmaster Monsanto Riverdale Thompson-Hayward	**H**		2,4,5-Trichlorophenoxyacetic acid 	Tan powder m.p. 152 C	EC WSC	100 (dogs)	Brush control on rangeland, pine tree stands, rights-of-way, aquatic weeds

CHAPTER 8

Teratogenicity of Pesticides

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SUMMARY AND CONCLUSIONS

Teratology deals with the etiology and development of congenital malformations. Congenital malformations are generally defined as gross structural abnormalities of prenatal origin, present at birth or manifesting shortly after, which kill or disable. In a broader sense, teratogenesis is considered to include histological, biochemical, and functional abnormalities of prenatal origin.

Congenital malformations present obvious personal, medical, and social stresses. Additionally, it has been recently estimated that the costs to society of one severely malformed child, in terms of medical and other care and deprivation of potential earnings, amount to several hundred thousand dollars.

There are now well over 400 substances that, in various forms and combinations, are currently used as pesticides. Pesticides may represent an important potential teratogenic hazard. Therefore any teratogenic pesticide to which the population is exposed should be promptly identified so that appropriate precautions can be taken to prevent risk of human exposure. It is feasible to test these substances for teratogenic effects in test animals so that potential hazards to human health can be evaluated.

For these and other reasons detailed in the report, we conclude that:

a. All currently used pesticides should be tested for tetratogenicity in the near future in 2 or more mammalian species chosen on the basis of the closest metabolic and pharmacologic similarity to human beings possible. Pesticides should be tested at various concentrations including levels substantially higher than those to which the human population are likely to be exposed. Test procedures should also reflect routes related to human exposure. Apart from the obvious route of ingestion, attention should be directed to other routes of exposure, including inhalation exposures from pesticide aerosols and vaporizing pesticide strips used domestically and exposures from skin absorption. Parenteral administration is an appropriate test route for pesticides to which humans are exposed by inhalation, or for pesticides which are systemically absorbed following ingestion.

b. The use of currently registered pesticides to which humans are exposed and which are found to be teratogenic by suitable test procedures in one or more mammalian species should be immediately restricted to prevent risk of human exposure. Such pesticides, in current use, include Captan; Carbaryl; the butyl, isopropyl, and isooctyl esters of 2,4-D Folpet; mercurials; PCNB; and 2,4,5-T. The teratogenicity of 2,4-D, the other salts and esters of both 2,4-D and 2,4,5-T, and that of IPC should be investigated further.

c. Pesticides found to be inactive after appropriate testing can be considered as provisionally safe, unless other evidence of teratogenicity develops.

d. No new pesticide should be registered until tested for teratogenicity by suitable procedures. Any pesticide found to be teratogenic should only be used in circumstances where risk of human exposure is minimal.

e. Efforts should be made to improve and standardize procedures for teratogenicity testing and population monitoring.

A scientific group or commission should be charged with responsibility for continued surveillance of the whole problem of pesticide teratogenesis.

METHODOLOGIES FOR TERATOGENICITY TESTING

Introduction

Prior to 1963, the Food and Drug Administration did not require evaluation of teratogenicity. As a result of the thalidomide disaster, the need for data on teratogenicity became evident. In 1963, the President's Science Advisory Committee on "Use of Pesticides" recommended that toxicity studies on pesticides include effects on reproduction through at least 2 generations in at least 2 species of warmblooded animals. Observations to be included were effects on fertility, size and weight of litters, fetal mortality, teratogenicity, and growth and development of sucklings and weanlings. Such toxicity studies including the three-generation procedure were not designed primarily to detect teratogenicity and thus may not be appropriate.

The potential teratogenicity of chemicals may be detected by two complementary approaches. First, chemicals or other agents may be administered to experimental animals to determine whether they induce prenatal damage. Secondly, and on a *post hoc* basis, human populations may be epidemiologically surveyed to detect geographical or temporal clusters of unusual types or frequencies of congenital malformations. Combinations of these approaches are likely to ensure early detection and identification of teratogenic hazards.

Experimentally, a complex of factors are needed to elicit teratogenic effects. These relate to gestation period, genotype of the pregnant animals, dosage, mode of administration and metabolic transformation of teratogen. For example, teratogens may be effective only at a certain dose range, whether high or low, narrow or wide, below which development is apparently undisturbed, and above which death *in utero* results.

Most agents are teratogenic only in the developmentally labile early period of gestation, during which active organogenesis occurs. In humans, this sensitive period extends aproximately from the end of the first week of pregnancy to the 12th week. Other circumstances may also influence the effectiveness of human teratogens, such as maternal nutritional, demographic, socioeconomic, and cultural factors, physiological states, and temporal and seasonal situations. Thus a potential teratogen may manifest its effect only when particular conditions conjoin.

The relationship between human exposure to a teratogen and subsequent induction of congenital abnormalities is generally not obvious. Any one teratogen may produce a multiplicity of effects and any specific effect may be produced by various teratogens. In test animals, the teratogenetic response may differ from species to species. In humans, differences in genetic, metabolic, and environmental influences may contribute to a variety of specific effects from exposure to a particular teratogenic agent. Induced and spontaneous effects may be difficult to distinguish. The teratogenicity of thalidomide might have been missed had it not produced malformations rarely encountered; additionally, only a fraction of the pregnant women who took thalidomide had defective children.

Consequently, further data on the possible teratogenic effects of pesticides in experimental animals are urgently needed to provide a basis for evaluating potential hazards to human health.

Ancillary methods

Preliminary screening can be accomplished by the use of nonmammalian species, particularly the chick embryo. These tests may give useful ancillary data prior to further testing in mammals. However, negative results in these systems alone should not be considered proof of safety.

Use of lower mammalian species

a. Purity, composition, stability, and source of compounds under test should be determined.

b. At least two mammalian species should be tested. These should be chosen on the basis of metabolic and pharmacokinetic similarity to humans. If possible, commercially available inbred strains should be used; if not, intra-species variability must be recognized. Species commonly used include mice, rats, hamsters, rabbits, dogs, cats; sheep and swine have also been used.

c. Preliminary mammalian experiments should determine the amounts of the compound and its appropriate metabolites necessary to produce serum levels comparable to ranges likely to be found in humans after high level accidental exposure as well as potential exposures assuming extensive use of that pesticide. Multiples of these dosages, up to the mammalian maternal LD_{50} should be administered to determine the lowest dosage causing a significant increase in fetal death, or resorption. Dosage in this critical range should be tested for teratogenic effects with care to distinguish these effects from other embryotoxicity and to determine dose-response relationships.

d. Compounds should be administered, by appropriate routes, within the critical dose range determined by preliminary tests. Parenteral administration is an appropriate test route for pesticides to which humans are exposed by inhalation, or for pesticides which are systemically absorbed following ingestion. Compounds should first be tested by single administrations of a range of doses at various times during the phases of active organogenesis. The substance should be administered at discrete times throughout the period of organogenesis as various organs are developing, since some substances have specific effects on the development of particular organs. By this technique, the possibility of inducing hepatic microsomal or other enzymes facilitating metabolic detoxification or activation of the substance is also minimized. If no teratogenic effects are detected by this technique, subsequent testing should be based on repeated administrations of the substance at daily intervals or if feasible, intervals of less than 24 hours during the entire period of organogenesis.

e. When appropriate, metabolites should also be tested for teratogenic effects.

f. Additional investigations should include—

i. Determination of appropriate plasma and fetal levels of compounds;

ii. Determination of the biological half-life of the compound in test animals;

iii. Metabolic studies to identify mechanisms of detoxification or activation of compounds when appropriate; and

iv. Determination, when appropriate, of the possible potentiating effects of protein deprivation or concomitant exposure to other pesticides or other environmental agents.

g. All procedures, including those relating to animal breeding, housing, handling, feeding, husbandry, methods for examining fetuses for congenital malformations, defining the onset of pregnancy, and classifying congenital malformations should be rigorously standardized. Numbers of pregnant animals and offspring must be adequate for statistical significance. All tests must be replicated on independent occasions and with contemporaneous controls.

Nonhuman primates .

Results from lower mammalian species may warrant subsequent testing in nonhuman primates The following considerations should be noted:

a. Records of menstrual cycles are essential. Primates whose reproductive history is known and have previously delivered normal young should be selected for testing. Timing of ovulation, and therefore gestation, should be accurately determined by allowing the males and the females to be together for no more than 3 consecutive days. Vaginal smearing, to determine the presence of spermatozoa should be avoided; the use of Tullner's method for determining chronic gonadotropin levels and rectal palpation is preferable.

b. Compounds should be carefully administered in controlled dosages.

c. Pregnant animals should be handled only minimally.

d. Compounds should be administered during the various phases of organogenesis. Embryos can be obtained by laparotomy any time after the first 100 days of gestation; the mother may be subsequently used for other experimental procedures. Additionally, some young should be allowed to go to term to identify possible teratogenic effects detectable only in the neonatal period.

Population monitoring

It has been shown (see Literature Review) that some pesticides induce congenital malformations in experimental animals providing a critical dose is appropriately administered at critical times. When animal experiments indicate that a pesticide is teratogenic, human effects should be retrospectively evaluated, when possible, by study of pregnancies during which the mothers were inadvertently exposed to the pesticide, such as a result of farm work, accidental ingestion, or industrial exposure. Prospective epidemiologic approaches may involve follow-up of large numbers of people over long periods of time, and be slow, tedious, expensive, or difficult to implement. It is not appropriate to conduct prospective epidemiological studies on human populations with pesticides previously shown to be teratogenic by experimental animal studies or retrospective human data. Human exposure to such compounds must be minimized by appropriate regulatory preventive action.

Prospective epidemiological approaches for pesticides in current use may provide important information, however, it should be realized that no major teratogen has yet been recognized in this way. The malformations induced by X-ray, German measles, thalidomide, and mercury—Minamata disease, were each recognized by an alert medical practitioner who observed a cluster of cases and then traced the cause to its source.

What can be done to enhance prompt recognition of such clusters should they occur from previously unsuspected teratogens in the future? A variety of existing data resources can be used for this purpose. In each, the occurrence of congenital malformations in substantial segments of the population is being recorded in a standard fashion. The best of these resources are local, rather than statewide or national. The prepaid medical program of the Kaiser-Permanente Hospitals and Clinics in the San Francisco Bay Area are of particular interest. A detailed study there of the occurrence of malformations among 16,000 births represents a good model for additional investigations. A similar study has been made by the Health Insurance Plan of Greater New York, but its 30 or more cooperating clinics are less easily coordinated than the Kaiser system.

A citywide surveillance, known as the Metropolitan Atlanta Congenital Defects Program (jointly directed by Emory University School of Medicine, the Georgia Department of Public Health, and the National Communicable Disease Center, USPHS), involves reports on all children with congenital malformations born to residents of the five-county Atlanta area. As yet, no cluster of cases has suggested an environmental influence since the program began in October, 1967.

In a substantial number of States, birth certificates contain an item concerning congenital malformations. The completeness and accuracy of such reporting varies considerably and depends on the physician's interest and diligence and on the conspicuousness of the abnormality. Birth-certificate data on malformations in New York State are more extensive than those of many other States and have been effectively used in several research studies. Nationally, however, no attempt has been made to collect and evaluate all data on malformations that are available on birth certificates.

A select committee convened by the National Center for Health Statistics (NCHS), has recommended, in an excellent but little known report, that efforts be made to improve and use information on congenital malformations recorded on birth certificates (Vital and Health Statistics, Documents and Committee Reports, NCHS Series 4, Number 7, March 1968). Implementation of this recommendation would be of great value, for monitoring to detect the teratogenic effects of newly introduced or geographically localized environmental chemicals or other agents.

To enhance our ability to recognize significant changes in congenital malformation rates, a systematic collection of data from concentration points should be established. Specifically, a surveillance should be made of claims submitted to private, State, or local agencies for the medical care of children with birth defects. Because the Children's Bureau, DHEW, has so much experience with these agencies, its assistance should be sought in planning the surveillance network.

Data from foreign countries should also be evaluated as part of a national effort to study possible relationships between pesticides and congenital malfunctions.

In studying the possible relationships between exposure to pesticides and the occurrence of diseases, statistical associations, if present, will provide important information. However, when possible it is important to secure additional information concerning the following:

a. Dose-response relationships.
b. Absence of alternative explanations.
c. Biological plausibility.
d. Consistency with other knowledge from clinical, laboratory, and epidemiologic research.
e. Disappearance of the effect when the presumed cause is removed.

In particular, as clusters of specific anomalies are recognized, through whatever resources that presently exist or may be developed, any possible relationships to pesticides would be clarified by the use of laboratory techniques to measure the maternal, fetal, or neonatal body burden of suspect chemicals.

There are national units engaged in teratologic research, but each is following a set method. There is a critical and immediate need to establish a national or international center to study congenital malformations in man not by a single method but by whatever techniques are most appropriate for testing or generating hypotheses. The center should be diversified and fast moving, ready to use local, national, or international resources in order to determine the significance of laboratory or clinical data.

LITERATURE REVIEW

Animal studies

For convenience, detailed results of the Bionetics study are presented in a subsequent section.

Much of the total available literature and data reviewed by this Panel were methodologically inadequate to support definitive conclusions. Additionally, the authors of many reports tended to confuse or equate embryotoxicity and other adverse effects on reproduction with teratogenicity. It is also apparent from the literature that insufficient attention has been directed towards problems of interactions in testing for teratogenesis.

The Panel considered the following information to be of significance:

a. Captan and Folpet.—These pesticides have been shown to be teratogenic in chicken embryos (Verrett et al., 1969). Captan was also shown to be teratogenic in rabbits (McLaughlin, 1969), although other rabbit studies yielded negative results (Kennedy et al., 1968; Fabro et al., 1965). The enhancement by protein deprivation of the acute toxicity of captan to rats (Boyd, 1968), was noted with particular interest. The teratogenicity of captan and Folpet in mice was demonstrated in Bionetics studies. Unpublished data on captan in monkeys were evaluated and found inadequate; in these studies, the duration of organogenesis was not entirely covered and controls were not appropriate. However, the 3/7 abortions observed at the highest dosage given, 25 mg./kg., may be indicative of an embryotoxic hazard due to captan.

b. Carbaryl.—This was tested at 66.7 and 200 p.p.m. in the diet of pregnant mice (FAO/WHO, 1967). In two litters at the 200 p.p.m. level, a total of seven instances of skeletal malalignment, nonfusion, incomplete ossification, and one case of cleft palate and gross facial malformation were noted, as opposed to no malformations in the low-level group and two cases of cleft palate in controls. Teratogenetic findings for carbaryl are also reported in the Bionetics study. In a study in beagle dogs fed carbaryl during gestational periods at levels of 50, 25, 12.5, 6.25, and 3.125 mg./kg. body weight daily, teratogenic effects were found at all but the lowest dose level (Smalley, 1968).

c. Mercurials.—Organomercury compounds: Various mercury containing pesticides were evaluated under the heading "phenylmercury acetate (and other organomercury compounds)" by the 1966 Joint Meeting of the FAO Working Party and the WHO Expert Committee on Pesticide Residues (FAO/WHO, 1967). The results of additional experimental work have been reported in the 1967 Evaluations of Some Pesticide Residues in Food. Additional information on "Methylmercury" was published by the Ecological Research Committee, the Swedish Natural Science Research Council (1969) Bulletin no. 4. by Goran Lofroth, where embryotoxic effects in mice (reported by Frölén and Ramel) were discussed along with other data. When given subcutaneously, in doses of 0.11 mg. on day 7 of gestation, phenylmercuric acetate was reported to cause fetal malformations in mice. Eye, tail, and central nervous system defects were noted (Murakami *et al.*, 1956).

d. Organochlorine.—Embryotoxicity in rats and dogs has been reported for organochlorines including dieldrin, chlordane, and kepone. In the absence of convincing data, kelthane has been claimed to be teratogenic in mice (An Der Lan, 1964); see also Bionetics studies.

e. Organophosphates.—The cholinesterase-inhibiting organophosphate insecticides, guthion, parathion, diazinon, Bidrin, Trithion, and EPN, have been shown to be teratogenic when injected directly in the yolk sac of chick embryos. The malformations were nonspecific or common to all organophosphates (Fish, 1966). It was also claimed that these compounds are teratogenic in mice. The data reported, however, suggested that organophosphates, like the organochlorines, act by reducing litter size and producing embryotoxicity rather than by producing specific teratogenic effects. See also Bionetics studies.

f. Thiram.—Thiram was reported to be teratogenic in hamsters at 250 mg./kg. (Robens, 1969). In the Bionetics study it was not found to be teratogenic. In a study of three generations of rats, no toxicological effects were observed at a dietary level of 48 p.p.m. (FAO/WHO, 1967). However, Thiram should be further investigated for possible teratogenic effects.

g. Miscellaneous reproductive effects.—Placental transfer of dieldrin and incidence of stillbirths have been studied in cows (Braund, 1968); increased stillbirth rates have been claimed in cows fed with DDT (Labon, 1965). The estrogenic activity of o,p'DDT has been related to reproductive effects in chicken, quail, and rats (Bateman, 1968, Wurster, 1968; Porter and Weimeyer, 1969). Diminished population size and reproductive failure have been produced in sparrow hawks by DDT and dieldrin (Porter and Weimeyer, 1969). These resulted from a decreased eggshell thickness, increased breakage of eggs, and increased egg eating by parent birds. Other studies of interest include the following: Finnegan, 1949; Tauber, 1950; Fisher, 1952; Narpozzi, 1956; Swann, 1958; Cottrell, 1959; Marliac, 1964; Backstrom, 1965; Hathaway, 1967; Ware, 1967; Weike, 1967; Carlton, 1968; Keplinger, 1968; Khera, 1968; Verrett, 1969; Legator, 1969.

Bionetics animal studies

Bionetics Research Laboratories of Litton Industries, during 1965–68 under a contract for the National Cancer Institute (NCI Contracts PH 43-64-57 and PH 43-67-735), tested various pesticides and related compounds for teratogenic effects. These studies were designed as large-scale screening tests. The Bionetics data were reanalyzed statistically to account for litter effects. The results of this statistical re-evaluation are presented in this section. More detailed material on these pesticides will be published in the future.

a. Summary of findings from Bionetic animal studies.—Tested more extensively than other pesticides, 2,4,5-T was clearly teratogenic as evidenced by production of statistically increased proportions of litters affected, and increased proportions of abnormal fetuses within litters in both DMSO and Honey for both C57BL/6 and AKR mice. In particular, cleft palate and cystic kidneys were significantly more prevalent. In addition, a hybrid strain resulting from a C57BL/6 female and AKR male showed significant increases in anomalies, in particular cystic kidney, when administered at 113 mg./kg. of body weight in DMSO.

Additionally, 2,4,5-T was tested in Sprague-Dawley rats. When given orally at dosages of 4.6, 10.0 and 46.4 mg./kg. on days 10 through 15 of gestation, an excessive fetal mortality, up to 60 percent at the highest dose, and high incidence of abnormalities in the survivors was obtained. The incidence of fetuses with kidney anomalies was three-fold that of the controls, even with the smallest dosage tested.

PCNB produced an increase in renal agenesis between litters, and within litters, when administered orally from days 6–14 or days 6–10 of pregnancy. However, renal agenesis was not produced when PCNB was administered only from days 10–14 of pregnancy. These effects were produced in only the C57BL/6 strain of mice.

Other pesticides producing a statistically significant increase in the proportion of litters containing abnormal fetuses and in the increased incidence of abnormal fetuses within litters were: Captan, Folpet, 2,4-D isooctyl ester, 2,4-D butyl ester, 2,4-D isopropyl ester, carbaryl (Sevin), and IPC. These pesticides produced elevated inci- and in one solvent only. The results for carbaryl and for IPC were less consistent than for other compounds. (The pesticides 2,4,5-T, PCNB, captan, Folpet, carbaryl, IPC, and the butyl and isopropyl esters of 2,4-D were statistically significant at the .01 level, for one or more tests. This criterion is similar to that adopted by the Technical Panel on Carcinogenesis, Chapter 5, to identify "positive" compounds. The isooctyl ester of 2,4-D was significant at the 0.05 level.)

Compounds inducing only an increase in the proportion of abnormal fetuses within litters were: a-naphthol, and 2,4-D methyl ester. The statistical significance of these results was relatively weak; further study is required before any conclusions can be reached. Similarly, 2,4-D produced only an increase in the proportion of abnormal litters during 1965 in AKR mice. Due to the teratogenic activity of certain of its esters, 2,4-D should be studied further.

Carbaryl plus piperonyl butoxide did not show an overall increase in nonspecific anomalies, but resulted in significantly more cystic kidneys for doses above 10 mg./kg. carbaryl plus 100 μl./kg. piperonyl butoxide.

It must be emphasized that failure to detect statistically significant increases of anomalies may be due to insensitivity resulting from experimental variation and small numbers of litters tested. In addition, higher fetal mortality among some of the "negative" compounds may be selectively eliminating abnormal fetuses.

b. Methods.—Four strains of mice were used: C57BL/6, AKR, C3H, and A/Ha. Most of the studies were performed with the C57BL/6 strain. A hybrid fetus resulting from mating a C57BL/6 female with an AKR male was used to study a few compounds. More restricted studies were also made on Sprague Dawley rats; results of these with reference to 2,4,5-T are considered separately.

Most compounds were administered subcutaneously in 0.1 ml. solutions of dimethylsulfoxide (DMSO). Water soluble compounds were administered in saline, and some times also in DMSO. Compounds administered orally were given by gavage in 0.1 ml. in a 50-percent honey solution. Groups of positive controls and untreated controls were included, as well as controls receiving only DMSO, saline, or honey. While controls were run periodically throughout the duration of the study, compounds and controls were not matched with respect to either route or date of administration.

Virgin females were used in these studies. The onset of pregnancy was determined by detection of vaginal plugs. Compounds were administered daily from the sixth to the 14th day of pregnancy (15th day for AKR mice). Mice were sacrificed on the 18th day (19th day for AKR mice) of gestation. On sacrifice, fetuses were examined for anomalies. Approximately two-thirds of the fetuses were then stored in Bouin's solution until necropsy. Remaining fetuses were stained with alizarin red S after proper processing. Numbers of resorption sites and dead fetuses were also scored.

c. Statistical analysis.—All analyses were performed on a *per* litter basis rather than a *per* fetus basis, since initial investigations indicated that the occurrences of anomalies among fetuses within litters were correlated. The large litter-to-litter variation may reflect some maternal effect, an indication of the effective dose level of the compound actually reaching the fetuses, experimental variation, or, as is most likely, some combination of the three factors.

While there were no statistically significant time trends within the various control groups in terms of the onset of fetal anomalies in the C57BL/6 mice, the incidence of fetal mortality was certainly time-dependent in this strain, with 1965 being characterized by a low incidence of prenatal deaths. Furthermore, there was a period of approximately 6 months, extending from the latter part of 1965 into early 1966, during which no control animals were tested. During this period a change in the substrain of C57BL/6 mice used in the study took place. Finally, among abnormal litters, as defined by litters containing at least one abnormal fetus, there was some suggestion that the distribution of abnormal fetuses *per* litter was stochastically larger in the DMSO controls than it was in the untreated controls. Thus, the possibility exists of a time/strain/solvent interaction that is undetectable in the controls because the level of background teratologic activity is relatively low. This potential interaction effect could either enhance or dissipate the effect of any given compound, depending on the conditions under which it was administered. Thus, the data were necessarily separated by both time period and solvent for the purposes of analysis. Similarly, an increase in fetal anomalies in the DMSO controls of the AKR mice was noted after November 1966. Thus, the AKR data were analyzed separately in two time periods.

It should be noted that not all compounds were administered on more than one occasion or in more than one solvent or strain. Thus, in general the compounds in the study cannot be compared for teratogenic potential, since those that were tested extensively were more likely to show some adverse effect and, perhaps, less likely to appear consistent over time, solvent, and/or strain.

As noted, approximately two-thirds of the fetuses were stored in Bouin's solution until necropsied; the remainder being stained with alizarin red. However, in many instances the proportion of necropsied fetuses was slightly higher for the compound under investigation than for the corresponding controls. It is doubtful if this discrepancy could have any appreciable effect on the conclusions since the incidence of anomalies detectable only by necropsy among control animals was relatively low. Furthermore, if all of the control and test mice had been necropsied, the significance of the differences observed in this study would be intensified. Thus, no effort was made to correct for inequalities in the necropsy/stain ratio in the present analysis. Additionally, no attempt was made to correct for differences in litter sizes or sex-ratios within litters, since both of these factors may, at least in part, reflect effects of the compound under test.

d. Results.—Data for pesticides yielding a statistically increased level of anomalies in C57BL/6 and AKR mice are listed in tables 1 and 2, respectively. The proportion of abnormal litters gives the proportion of litters containing one or more abnormal fetuses, as a measure of the prevalence of anomalies across litters. The proportion of abnormal fetuses *per* litter gives a measure of the prevalence of anomalies within litters. The proportion of abnormal fetuses *per* litter for litters containing abnormal fetuses gives a measure of the prevalence of anomalies within effected litters. A significant increase of dead fetuses and resorptions is also listed. Some tests were conducted on only one particular day or on adjacent days as listed. Eye anomalies, mainly microphthalmia and anophthalmia, accounted for approximately 50 percent of the individual anomalies in C57BL/6 mice. To a large extent, results in table 1 reflect changes in the incidence of eye anomalies. Yet, when the data were analyzed excluding fetuses with microphthalmia only, there were no striking changes in the results. In the last column of table 1, statistically significant increase in various types of anomalies other than eye anomalies are listed. The positive controls, trypan blue and ethyleneimine, table 1, and 6-aminonicotinamide, table 2, showed elevated levels of anomalies, although the latter control did not yield consistent results over all dose levels.

Only those test conditions which resulted in statistically elevated incidences of anomalies are listed in tables 1 and 2. Some compounds gave no increase in anomalies (based on the overall incidence if tested in both time periods) when tested in other solvents, strains, or dose levels (table 3). It must be emphasized that failure to detect a statistically significant increase in anomalies may only be a reflection of experimental insensitivity due to experimental and biological variaton and insufficient number of litters. Thus, compounds showing no increases cannot be considered nonteratogenic. For example, trypan blue in DMSO at the highest dose level tested, 37.5 mg./kg., did not show an increase in anomalies, possibly due to higher fetal mortality. Standard corrected 2 × 2 chi-square tests (*1*) were used to compare the proportion of abnormal litters for the compound with the controls in the same solvent. In the cases where tests were conducted in two time periods, the results from the two chi-squares were combined (*1*). The levels of statistical significance for the combined tests are listed under the total column for proportion of abnormal litters.

The distribution of the proportion of abnormal fetuses per litter (tables 1 and 2) for compounds were compared with the appropriate control distribution by use of the nonparametric Mann-Whitney U-test (*2*). This test requires that the proportion of abnormal fetuses per litter is independent from litter to litter, but requires no assumption about the frequency distribution of these proportions. Again, where litters were run in both time periods, the significance level for the combined tests is given under the total column. Bracketed data include groups which were combined before statistical tests were conducted.

STATISTICAL REFERENCES

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TABLE 1.—*Tests which displayed significant increases of anomalies (C57BL/6 mice)*

Compound	Solvent	Dose per kg of body weight	Proportion of abnormal litters			Proportion of abnormal fetuses per litter			Proportion of abnormal fetuses per litter in abnormal litters			Increased mortality	Tests repeated over time	No. of live litters		Increased anomalies other than eye
			1965	1966–68	Total	1965	1966–68	Total	1965	1966–68	Total			1965	1966–68	
Negative controls:																
Untreated	None		.42	.39	.40	.08	.11	.10	.18	.28	.25			26	69	
Controls	DMSO		.53	.41	.46	.16	.12	.13	.33	.28	.29			70	112	
Do	Saline		.52	.37	.43	.13	.10	.11	.24	.28	.26			31	46	
Do	Honey			.47	.47		.15	.15		.32	.32				32	
Positive controls:																
Trypan blue	DMSO	5.0 mg	.60		.60	.32		.32	.54		.54	Yes		5		Hydrocephaly.
Do	DMSO	12.5 mg	.86		.86	.44***		.44***	.52**		.52**	Yes		7		
Do	DMSO	37.5 mg	.60		.60	.36		.36	.60		.60	Yes		5		
Do	Saline	5.0 mg	1.00		1.00	.61***		.61***	.61**		.61**			5		
Do	do	12.5 mg	.71		.71	.49**		.49**	.69***		.69***	Yes		7		
Do	do	37.5 mg	.71		.71	.33*		.33*	.46**		.46**	Yes		7		Hydrocephaly.
Ethyleneimine	do	4.64 µl	1.00*		1.00*	.49***		.49***	.49***		.49***	Yes	No	7		
Experimental:																
2,4,5-T	DMSO	113 mg		.79**	.79**		.56***	.56***		.71***	.71***	Yes		14		Cleft palate cystic kidney.

2.4.5-T	Honey	46.4 mg	----------	1.00*	1.00*	----------	.37**	.37**	----------	.37	.37	No	6	------	
2.4.5-T	do	113 mg	----------	1.00**	1.00**	----------	.70***	.70***	----------	.70***	.70***	Yes	9	------	Cleft palate cystic kidney.
PCNB (days 6-14)	do	215 mg	----------	.88*	.88*	----------	.25**	.25**	----------	.29	.29	No	8	------	
PCNB (days 6-14)	do	464 mg	----------	.67} **	.67} **	----------	.25} ***	.25} ***	----------	.38	.38	----------	12	------	Renal agenesis.
PCNB (days 6-10)	do	464 mg	----------	1.00}	1.00}	----------	.38}	.38}	----------	.37	.37	No	10	------	
Captan	DMSO	100 mg	1.00*	.61	.71***	.58***	.27	.35***	.58**	.44	.49 **	Yes	6	18	
Folpet	DMSO	100 mg	----------	.77 **	.77 **	----------	.29 ***	.29 ***	----------	.38 *	.38 *	----------		13	
2,4- Isooctyl ester	DMSO	48 µl	1.00 *	----------	1.00 *	.24	----------	.24	.24	----------	.24	----------	6	------	
2,4-D Isooctyl ester	DMSO	130 µl	----------	.67	.67	----------	.28 **	.28 **	----------	.41 *	.41 *	----------		15	
2,4-D Butyl ester	DMSO	100 µl	----------	.75 **	.75 **	----------	.25 ***	.25 ***	----------	.34	.34	----------		20	Agnathia.
2,4-D Isopropyl ester.	DMSO	94 µl	----------	.70 **	.70 **	----------	.26 ***	.26 ***	----------	.37 *	.37 *	----------		20	
Carbaryl	DMSO	100 mg	1.00 *	.54	.71 **	.46 ***	.16	.26 **	.46 *	.29	.37	----------	6	11	Hydrocephaly, skeletal
IPC	DMSO	850 mg	1.00 **	.43	.71 *	.46 ***	.09	.27 **	.46 *	----------	.46 *	----------	7	7	
α-Napthol	DMSO	10 mg	.86	----------	.86	.33 *	----------	.39 *	.38	----------	.38	----------	7	------	
2,4-D Methyl ester	DMSO	106 mg	----------	.83	.83	----------	.30 *	.30 *	----------	.36	.36	----------		6	
Carbaryl+Piperonyl Butoxide.	DMSO	10 mg+ 100 µl	----------	.50	.50	----------	.13	.13	----------	.26	.26	No		6	Cystic kidney
Do	DMSO	46.4 mg+ 464 µl	----------	.50	.50	----------	.10	.10	----------	.21	.21	----------		12	

Significance level: *(.10). **(.05). ***(.01).

TABLE 2.—*Tests which displayed significant increases of anomalies (AKR mice)*

Compound	Solvent	Dose per kg of body weight	Proportion of abnormal litters			Proportion of abnormal fetuses per litter			Proportion of abnormal fetuses per litter in abnormal litters			Increased mortality	Tests repeated over time	No. of live litters		Special anomalies
			†11/66	12/66††	Total	†11/66	12/66††	Total	†11/66	12/66††	Total			†11/66	12/66††	
Negative controls:																
Control	DMSO		.05	.37	.21	.01	.06	.03	.11	.16	.15			37	35	
Do	Honey			.00	.00		.00	.00							12	
Positive controls:																
6-amino-nicotinamide.	DMSO	.34 mg	.56 ***		.56 ***	.31 **		.31 **	.55		.55			9		Cleft palate.
6-amino-nicotinamide (1).	DMSO	.68 mg	.00		.00	.00		.00						7		
Experimental:																
2,4,5-T	DMSO	113 mg	.50 ***	1.00 **	.71 ***	.20 **	.40 ***	.29 ***	.40 *	.40 **	.40 *			8	6	Cleft palate
2,4,5-T	Honey	113 mg		1.00 ***	1.00 ***		.54 ***	.54 ***		.54	.54	yes			6	Cleft palate
2,4-D	DMSO	98 mg	.43 **	.29	.36 *	.12	.05	.08	.28	.16	.23			7	7	

*Significance Level .10. **Significance Level .05. ***Significance Level .01.
†Through 11/66 ††After 11/66
Note: (1) With the .68 mg/kg dose, as compared to the .34 mg/kg dose, fewer implantations and a higher fetal mortality were encountered, resulting in fewer live fetuses per litter.

TABLE 3.—*Tests which showed no significant increase of anomalies (with particular doses, solvents, or test strains)*

Compound	Strains	Solvent	Dose per kg. body wt.	Increased mortality (C57BL/6)	Total number of litters
2,4,5-T	C57	DMSO	21.5 mg.	--------	6
PCNB (days 10–14)	C57	Honey	464 mg.	--------	9
PCNB	AKR	Honey	464 mg.	--------	9
Captan	C57	Honey	100 mg.	--------	12
Do	AKR	DMSO	100 mg.	--------	13
Folpet	C57	Honey	100 mg.	--------	5
Do	AKR	DMSO	100 mg.	--------	13
2,4-D Isooctyl ester	C3H	DMSO	48 μl.	--------	6
Do	A/Ha	DMSO	24 μl.	--------	5
Do	AKR	DMSO	130 μl.	--------	8
2,4-D Butyl Ester	C57	DMSO	46 μl.	--------	6
Do	AKR	DMSO	100 μl.	--------	10
2,4-D Isopropyl Ester	C57	DMSO	46 μl.	--------	6
Do	AKR	DMSO	94 μl.	--------	6
Carbaryl	C3H	DMSO	100 mg.	--------	8
Do	C57×AKR	DMSO	100 mg.	--------	6
Do	AKR	DMSO	464 mg.	--------	13
IPC	C3H	DMSO	850 mg.	--------	11
IPC	AKR	DMSO	850 mg.	--------	13
2,4-D Methyl Ester	AKR	DMSO	106 mg.	--------	7
Do	C57×AKR	DMSO	106 mg.	--------	5
o,p′-DDD	C57	DMSO	100 mg.	--------	13
Do	AKR	DMSO	100 mg.	Yes	12
2,4-D	C57	DMSO	100 mg.	--------	16
Do	C57	Honey	100 mg.	--------	12
Do	C3H	DMSO	100 mg.	--------	6
Do	C57× AKR	DMSO	98 mg.	--------	11
Zectran	C57	DMSO	10 mg.	--------	7
Do	AKR	DMSO	10 mg.	--------	7
Thiram	C57	DMSO	10 mg.	--------	8
Do	AKR	DMSO	115 mg.	--------	7
Ferbam	C3H	DMSO	4.64 mg.	--------	6
Do	C57	DMSO	4.64 mg.	--------	6
Monuron	C3H	DMSO	215 mg.	--------	7
Do	C57	DMSO	215 mg.	--------	13
Do	C57	Honey	215 mg.	--------	9
Do	AKR	DMSO	215 mg.	--------	13
Diuron	C3H	DMSO	215 mg.	--------	6
Do	C57	DMSO	215 mg.	--------	6
2,4-D Ethyl Ester	C57	DMSO	86 μl	--------	7
Do	AKR	DMSO	86 μl	--------	7
Atrazine	C3H	DMSO	46.4 mg.	--------	6
Do	C57	DMSO	46.4 mg.	--------	13
Do	AKR	DMSO	46.4 mg.	--------	15

TABLE 3.—*Tests which showed no significant increase of anomalies (with particular doses, solvents, or test strains)*—Continued

Compound	Strains	Solvent	Dose per kg. body wt.	Increased mortality (C57BL/6)	Total number of litters
Piperonyl Butoxide	C3H	DMSO	1000 µl	--------	6
Do	C57	DMSO	1000 µl	--------	6
Do	C57	DMSO	21.5 µl	--------	6
p,p′-DDD	C57	DMSO	46.4 mg.	--------	6
p,p′-DDT	C57	DMSO	46.4 mg.	--------	6
Carbaryl + Nicotinamide	C57	DMSO	100+61 mg.	--------	10
Nicotinamide	C57	DMSO	61 mg.	Yes	6
CIPC	C57	DMSO	1000 mg.	--------	6
Nabam	C3H	DMSO	21.5 mg.	--------	6
Do	C57	DMSO	46.4 mg.	--------	6
Do	C57	Saline	46.4 mg.	--------	14
Do	AKR	DMSO	46.4 mg.	--------	5
Do	AKR	Saline	46.4 mg.	--------	14
Propazine	C3H	DMSO	464 mg.	--------	6
Dicryl	C57	DMSO	21.5 mg.	--------	6
Perthane	C57	DMSO	100 mg.	--------	6
Ovex	AKR	DMSO	185 mg.	--------	7
Tedion	AKR	DMSO	217 mg.	--------	6
Amitrol	C57	Saline	464 mg.	--------	13
Do	C57	Honey	215 mg.	Yes	8
Do	AKR	Saline	464 mg.	--------	14

Human studies

Epidemiologic data on possible effects of pesticides on human reproduction and teratology are grossly inadequate. Prospective studies on this subject are difficult to design and almost nonexistent, except for the community pesticide program of the Food and Drug Administration.

Chlorinated hydrocarbons.—In a recent review (Khera and Clegg, 1969), no adverse human reproductive effects were attributed to DDT and other chlorinated hydrocarbons. Studies on 240 pregnant women indicated that 21 percent had significant first trimester pesticide exposure, and that 52 percent were exposed during their entire pregnancy. No statistical difference in numbers of patients with anomalies existed between these exposed groups (Nora et al., 1967). Low values of DDT residues have been found in a small number of human placentas (Rappolt et al., 1969). Sharply reduced tissue levels were also found in 68 newborn infants (Zavon, 1969). Pesticide levels in human milk have not shown any relation to perinatal toxicity (Laug et al., 1951; Lofroth, 1969; Curley and Kimbrough, 1969). Studies on 152 mothers showed transplacental passage of DDT and DDE (O'Leary, 1969). Low placental and high vernix levels were noted; fetal blood levels were one-half maternal levels. In a similar study on premature infants (O'Leary, 1969), high fetal levels were noted; no relationship between maternal blood levels of DDE and DDT and the incidence of first trimester spontaneous abortion were found, although the number of pregnant women reported on was inadequate for firm conclusions.

Organophosphates.—Evidence of teratogenic potential of organophosphates in humans has been reviewed and found inconclusive (Khera and Clegg, 1969).

Mercurials.—Consumption by Japanese pregnant women of fish and shellfish contaminated by methylmercury produced a high incidence of infantile cerebral palsy (Matsumoto *et al.*, 1965). This condition has been termed fetal Minamata disease.

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